Vol. III MAY, 1918 No. 8

MAY 31 1918
UNIV. OF MICHIGAN LIBRARY

The Journal of Laboratory and Clinical Medicine

VICTOR C. VAUGHAN, M.D., Editor-in-Chief
University of Michigan, Ann Arbor

ASSOCIATE EDITORS

Pharmacology
DENNIS E. JACKSON, M.D.
Washington University, St. Louis

Bacteriology
HANS ZINSSER, M.D.
Columbia University, New York

Immunology and Serology
FREDERICK P. GAY, M.D.
University of California, Berkeley

Physiological Pathology
PAUL G. WOOLLEY, M.D.
University of Cincinnati, Cincinnati

Physiological Chemistry and Clinical Physiology
J. J. R. MACLEOD, M.B.
Western Reserve University, Cleveland

ROY G. PEARCE, M.D.
Lakeside Hospital, Cleveland

Clinical Microscopy and Laboratory Technic
ROGER S. MORRIS, M.D.
University of Cincinnati, Cincinnati

Neuropathology
E. E. SOUTHARD, M.D.
Harvard University, Boston

Tuberculosis
GERALD B. WEBB, M.D.
Cragmor Sanatorium, Colorado Springs

Published by
THE C. V. MOSBY COMPANY
ST. LOUIS

$3.00 Per Annum Single Copies, 30 Cents

Bacteriologic Peptone

PARKE, DAVIS & CO.

CHEMICALLY ANALYZED

CONTAINS:

Total Nitrogen	not less than 14.0%
Mineral matter	not more than 8.5%
Moisture	not more than 6.0%

Used in bouillon according to standard methods.

FURNISHES:

Diphtheria Toxin . of which 0.25 mil—1 L.+ Dose
Tetanus Toxin . . of which 0.001 mil—1 M. F. D.

BACTERIOLOGICALLY TESTED

Bacteriologic Peptone, P. D. & Co., is the one American-made peptone which fulfills the varied and exacting requirements of all bacteriologic work.

It has a maximum content of the proper amino acids, or protein "building stones."

It has the proper chemical structure for the production of diphtheria and tetanus toxins of highest potency.

Its efficiency has been established under practical working conditions by many of the best known scientists in North America. This work included, in addition to the routine of the bacteriological laboratory, such special procedures as cultivation of spirochætæ; analysis of milk, water and soil; standardization of disinfectants, manufacture of serums and vaccines, indol production tests, and some of the other more delicate biologic reactions.

Bacteriologic Peptone, P. D. & Co., is readily and completely soluble in all proportions, furnishing clear bouillon and agar.

Supplied in 1-pound bottles and 5- and 10-pound cans.

WRITE FOR LITERATURE AND QUOTATIONS.

Home Offices and Laboratories,
Detroit, Michigan.

Parke, Davis & Co.

The Journal of Laboratory and Clinical Medicine

VOL. III. ST. LOUIS, MAY, 1918 No. 8

ORIGINAL ARTICLES

THE PATHOLOGY OF THE SKIN LESIONS PRODUCED BY MUSTARD GAS (DICHLORETHYLSULPHIDE)*

BY ALDRED SCOTT WARTHIN, PH.D., M.D., AND CARL VERNON WELLER, M.D., ANN ARBOR, MICH.

THE great World War has been epoch-making in many ways and in no way more remarkable than in the introduction of poisonous gases as agents of warfare. First used with notable effects by the Germans in the Spring of 1915, they have since been employed by the Allied forces as well. Gas attacks have become frequent features of the conflict on both sides; and as a military agent poison gas may be regarded as more effective than the use of artillery. "Gassing" has come to be more feared than the missiles of warfare. Many kinds of poisonous gas have been used by the Germans, some of them, no doubt, more or less experimentally; but of all of those employed the most important one, both as to quantity and effective results, has been "mustard gas" (dichlorethylsulphide). This was first used on a large scale at Ypres, July 12-13, 1917; and at times thousands of shells containing hundreds of tons of this gas have been fired in relatively short periods. Exact data as to the results can not at present be made public.

Dichlorethylsulphide (thiodiglycolchloride),

$$S\begin{<}\begin{matrix}CH_2.CH_2.Cl\\ \\ CH_2.CH_2.Cl\end{matrix}\end{>},$$

was first made by Victor Meyer[1] in 1886. He described it as a heavy oily fluid sinking below water and not miscible with it, of neutral reaction, having a faint, sweetish, ethereal odor but slightly suggestive of the sulphur compounds, and

*From the Department of Pathology, University of Michigan, Ann Arbor, Mich.

with a boiling point of 217° C. He at once recognized the specific toxic properties of the substance from the fact that a laboratory worker engaged in making it, suffered from a severe skin eruption and a transitory conjunctivitis. As Meyer himself was not affected by exposure to the substance, he concluded that individual susceptibility to it must vary greatly. Animal experiments were carried out as follows: Two medium-sized rabbits were confined three to four hours in a closed cage ventilated by a moderately strong current of air passing through a glass tube containing strips of blotting paper saturated with thiodiglycolchloride. The animals became restless, rubbed their noses frequently with their feet. The nose and conjunctiva became reddened, and the eyes moist. Perspiration appeared to be increased. On the following day both eyes were severely inflamed, the lids glued together with purulent secretion. Marked snuffles developed, the lobes of the ears were much swollen, and the auditory passages showed a purulent inflammation. On the evening of the third day the animals died of a severe pneumonia diffused throughout both lungs. A strong rabbit that had inhaled the vapor of the substance for a few hours through a tracheal fistula, without exposure of the surface of the body to these vapors, died of a widespread pneumonia on the evening of the same day before other symptoms had appeared.

In rabbits in which the unbroken skin of the top of the ear had been touched with a fine brush containing a small amount of the chloride the site of the application showed no direct effects, but the entire ear became markedly swollen, and in one case a profuse purulent inflammation arose from the bottom of the auditory passage. In this case, the material could not have accidentally entered the auditory canal owing to the small amount used and the fact that it was applied to the outer surface of the ear. In one case in which the skin was scraped by the removal of the hair from the ear tip, the chloride applied by means of the brush caused an especially severe suppuration at this point, and also a marked swelling of the whole ear and inflammation of the eyes.

After subcutaneous injection of about two drops of the chloride into the back of a rabbit, there developed inflammation of both eyes, very severe snuffles, and on the third day death from pneumonia. At the point of injection no effects were apparent.

The experiments were discontinued because of similar unpleasant effects upon those engaged in the investigation. Meyer concluded that the most severe action of the chloride develops only after its entrance into the blood. Preliminary experiments with the glycol and the sulphide showed these to be non-poisonous.

In 1887, Meyer[2] stated that experimental animals surviving after application of thiodiglycolchloride (dichlorethylsulphide) to the ear, showed a persistent profuse suppuration and complete necrosis and falling off of the ear after a few weeks. Because of the marked toxic action of the dichlorethylsulphide, physiologic tests were made of the monochlorethylsulphide. This was found to possess poisonous properties like those of the dichlorethylsulphide, but less marked in intensity. In the comparison of these two substances and ethylsulphide, the chlorine-free sulphide was found to be harmless. The toxicity of

the mono- and dichlorethylsulphides, therefore, depends entirely upon the chlorine content.

From 1887 up to the last year, the poisonous nature of dichlorethylsulphide seems to have attracted little attention in either chemical or medical literature. Since its use as a poison gas in warfare, the name "mustard gas" has been almost universally applied to it because of the faint mustard-like odor; and from the war literature and war reports further knowledge of its toxic action has been gained.

The symptoms of mustard gassing are described in the British Army Reports as follows: Initial tendency to sneeze without irritation of eyes or lacrimation, with a gradually increasing nose and throat irritation, followed after about twelve hours by a free discharge of mucus from nostrils, painful irritation and inflammation of eyes, and occasionally vomiting. Many men had pain in forehead and stomach. Small blisters may form on face and neck; the skin between thighs is occasionally red, sore and sometimes blistered. Contact with fragments of shells and with the earth near shell holes may cause blistering through the clothes. None of these lesions manifests itself at once, they develop after several hours. The pain in the eyes may lessen after twenty-four hours, although acute inflammation may persist several days. In severe cases bronchitis and pneumonia may develop after thirty-six to forty-eight hours. There are usually no deaths under twenty-four hours. Prolonged exposure to small concentrations of the gas cause laryngitis and loss of voice, sufficient to put men out of action. Of the men affected practically all have conjunctivitis, about 95 per cent have throat and lungs affected, and about 70 per cent show skin burns. After six weeks the skin lesions are usually healed. The eye, pulmonary, and cardiac conditions recover more slowly.

Giraud,[3] in November, 1917, describes the early symptoms of mustard-gas poisoning, dividing them into three groups: eye lesions, respiratory tract lesions, and burns. All of these develop slowly, but the time of appearance varies for each lesion. The eye symptoms appear first, within six to twelve hours and are fully developed at the end of twenty-four to forty-eight hours. The respiratory affections usually appear at the end of two to four days. The skin lesions fall into two groups, the earlier burns, contemporaneous with the conjunctivitis or preceding it and later burns. The early burns are the most severe. The later burns appear at the end of four, five, eight, ten or even fifteen days. They are usually without danger. The three groups of lesions may be found singly or associated in the same individual. Giraud also finds that the eye lesions are most common, then follow those of the respiratory tract, and thirdly the burns.

Eye Lesions.—Conjunctivæ congested, vessels dilated, severe pain at times but usually moderate, moderate lacrimation, vision unimpaired, pupil reflexes normal, marked swelling of eyelids. The less severe cases heal in two to three days.

Lesions of the Respiratory Tract.—The initial symptom is aphonia which may come on suddenly or be preceded by a few paroxysms of coughing. It is usually complete, lasting three to four days. Tracheitis and bronchitis then

develop, the paroxysms of coughing are frequent and painful, closely simulating the cough of pertussis. At the end of two to three days, the patient discharges an abundant mucous or mucopurulent sputum. The affected patient can not breathe except in the erect position, and the majority decline to go to bed. Giraud has not observed hemoptysis. The respiratory affections are most intractable, and frequently relapse without any apparent reason.

Skin Lesions.—The early burns appear in twelve hours. These are the severe burns, presenting the appearance of large erythematous patches covered with large bullæ, containing a serous or seropurulent fluid. Occasionally, particularly on the scrotum, the large blisters are replaced by little purulent vesicles surrounding the roots of the hairs. *The pain is commonly quite severe,* and patients presenting burns of this degree are immediately evacuated. The lighter burns are more frequent, are insidious, appear slowly, but are the least serious of the mustard-gas lesions. Often they are unnoticed by the men, and are discovered only by chance. They appear as burns of the first degree in the form of erythematous plaques resembling sunburn. After a time, the central portion desquamates, leaving a slightly weeping surface without any actual blister formation. The burns appear most commonly around the joints of the lower extremities, thighs, genitals, buttocks, neck, and back. They are only exceptionally found upon the uncovered parts.

As to lesions of the digestive tract Giraud considers them exceptional, the frequent vomiting being attributable to efforts at coughing. He considers it probable, however, that lesions of the alimentary tract may be due to ingestion of food tainted by the gas.

All of the lesions observed by him have been purely local. He has only rarely seen signs of general disturbance, acceleration of pulse and fever, which he considers may have been due to other causes than the intoxication. The majority of the patients present an earthy pallor, but this is not surprising in men subjected to the fatigue of a prolonged stay in the first line trenches.

For prophylaxis and treatment he advises the use of a solution of bicarbonate of soda in a strength of thirty parts per thousand. Washing the eyes with the solution he found to be prophylactic against the conjunctivitis. For the burns he employs washing with the bicarbonate solution and a dressing of Vincent's powder (boric acid and calcium hypochlorite). The respiratory lesions he found resistant to treatment, which has been wholly symptomatic.

Teuliéres,[4] in the same month, November, 1918, describes the action of the gases used in warfare upon the visual apparatus. Under the heading of the "new gas," which he states is composed of carbon tetrachloride and ammonium-bichlorsulphide,* but described by the majority of soldiers as having a mustard odor, colorless, very heavy and impregnating the earth, and which, therefore, must have been dichlorethylsulphide, he says that the ocular lesions produced by it range from simple palpebro-conjunctival reddening up to the most severe burns of the conjunctiva and cornea. Since the gas seems to operate only in the presence of water, he advises against bathing the wounded. The treatment he advises is purely symptomatic.

*Corrected by author in erratum.

Mandel and Gibson[5] give a brief account of the clinical manifestations and treatment of mustard-gas poisoning, adding nothing to what has been gained from Giraud's article, except the statement that the myocardium, as demonstrated by necropsy, is not damaged. Their summary is as follows:

1. An interval (four to sixteen hours) of freedom from distress exists between the actual gassing and the onset of the symptoms.

2. The cardinal symptoms, conjunctivitis, laryngitis, bronchitis, and skin burns, are all due to the excoriating effect of the gas.

3. The principal complications are early pulmonary edema and relatively late bronchopneumonia.

4. Dry clothing and sleeping quarters may prevent the development of symptoms after slight exposure, and possibly may lessen the severity in those more severely gassed.

EXPERIMENTAL

Haldane[6] states that he has confirmed Meyer's statement that subcutaneous injection of the substance is capable of causing conjunctivitis and death from pneumonia, owing to the absorption of the gas into the circulation.

Kolls and Gilbert[7] report that 10 mg. of an alcoholic solution injected into the muscles and intravenously produced nothing more than temporary inactivity and loss of appetite, with recovery in one week; 50-100 mg. in alcohol and water emulsion produce convulsions within an hour and death in three hours. *Mice* exposed to the vapor after five minutes became depressed, developed irritation of nostrils and marked dyspnea. Except when high concentrations were used death was delayed two to six days. *Cats* exposed fifteen minutes to a concentration of 1:2000 showed lacrimation and restlessness; after two hours cat became wild, kept eyes closed; after twenty hours became dyspneic and died at thirty-six hours. A fifteen-minute exposure to a concentration of 1:10,000 gave similar results; the animal died in four days of pneumonia. In twenty hours photophobia and purulent conjunctivitis developed. *Rabbits* exposed for four hours to less than 1:100,000 concentration closed their eyes towards the end, developed well-marked conjunctivitis at end of twelve hours, and died in two days. After exposure to 1:20,000 concentration conjunctivitis developed in six hours, and was marked in twenty-four; there was marked dyspnea and snuffles after twenty hours.

Raper and Ball[8] found that rabbits exposed for five minutes to a concentration of 1:20,000 developed a very slight irritation of the eyes after five minutes; six hours later both eyes were kept closed, and there was a definite conjunctivitis which grew steadily worse with signs of nasal catarrh. When killed after four days there was found an intense congestion of the trachea and some patches of bronchopneumonia. A fifteen-minute exposure caused marked discomfort after ten minutes in the form of a marked irritation of eyes and nose; five hours later conjunctivitis and snuffles had developed. This was worse the following day, and the inflammation of the eyes became purulent. When killed on the fourth day similar changes to the above were found. Thirty-minute exposures produced the same results.

Marshall and Miller[9] worked with dogs. They found that higher concentrations (38 mg. per liter) caused immediate excitement, irritation of eyes and lacrimation, followed by dulling of cornea and depression. Frequent retching and vomiting was observed. At the end of exposure or shortly afterwards the mucous membranes were inflamed, and, in some cases, the skin on face and bare parts of hind legs became red. There was continued retching and vomiting, nasal discharge and salivation, dyspnea, tracheal rattle, and death in four to ten hours. With lower concentrations there was practically no sign during exposure. In twenty-four hours there developed conjunctivitis, nasal discharge, and tracheal rattle, and animals became depressed. There was no vomiting, as a rule. Death occurred in twenty hours to twelve days.

Kolls and Gilbert[10] showed that 1:100 alcoholic solutions act as skin irritants. With increasing dilutions up to 1:1000 the skin is killed, but there is little difficulty in healing. Further dilution produced no irritation. The skin lesions were found to show great individual variations.

Test tubes containing air saturated with mustard-gas vapor applied to the skin cause erythema in twenty-four hours after five minutes' exposure; after ten minutes' exposure erythema was more marked; after twenty minutes' exposure there developed in twenty-four hours a red papular area just short of a blister, with hyperesthesia. When the concentrated vapor is allowed to act on the skin for a short time a vesicle may not be produced, but only a red edematous patch with slight soreness on surface, something like a sunburn (Royal Society War Commission Reports).

One to two drops of a 1:100 solution in alcohol applied to the arm caused varying degrees of itching and blistering. Complete healing took place in seven to eight days, although a scar remained.

Other experimenters[11] find mustard gas to be a blistering agent of great power, producing erythema and blistering the skin as well as causing acute conjunctivitis, tracheitis and bronchitis. The intensity of the action seems to be increased by humidity, although if hands are washed immediately after exposure no lesions are produced. If the skin is touched with a glass rod just moistened with the gas and then wiped with cotton there results in two hours a red patch larger than the area touched. This increases in size, and at the end of four hours may be as large as a sixpence. After twenty to thirty hours a well-marked tense vesicle develops, with erythematous area around vesicle. If the latter is opened under aseptic precautions and infection is avoided, the lesion heals normally, but redness persists for three weeks or more. The whole process is painless.

Subjects exposed in chambers containing 1:10,000 (0.709 grams per M^3) of impure oil, when wearing French respirators, showed after six hours irritation of the skin, vomited and felt ill. On the next day there was marked erythema of the scalp, chest, inside of arms, hands, thighs and genitals. The erythema was more marked on one forearm that had been smeared with vaseline and lanolin. On the arm and on the genitals large blisters were produced.

Summary.—The general effects without protection in animals and in man were found to be conjunctivitis, sore throat, hoarseness, gradual necrosis of mucous membrane of air passages, bronchitis, later purulent in character, lead-

ing to bronchopneumonia, which may terminate in death, and more or less widespread burns of skin. These affect chiefly covered parts, but the face and hands may also show burns. The skin lesions develop as follows: Erythema, with some irritation or no pain, followed by blister, accompanied, particularly on the genitals, with much swelling and edema. The erythema is attended by diapedesis of red cells. Even when no blisters are formed, there may be dark purplish patches, the color of which does not disappear on pressure. Later these become pigmented dark-brown.

PATHOLOGY

Mackenzie[12] states the most important pathologic changes to be those found in the eyes, skin and respiratory system. In experimental animals there is diffuse clouding of the cornea at once which goes on to exfoliation of the corneal epithelium and to ulceration. The corneal vessels show a marked acute congestion; there is an abundant serous discharge, which later becomes purulent. The skin, where there is no hair, about the lips, nose, genitals, nipples and between toes, shows bright red discolorations.

The upper air passages show patches of intense hemorrhagic redness, in the mouth sometimes to the extent of ulceration, particularly at points of contact with teeth and cheeks. The pharynx, epiglottis and larynx are reddened. From the upper end of the trachea throughout the entire bronchial tree, the mucous membrane is covered with a thick translucent membrane of a pale yellowish color, which is easily pulled off in large strips. It extends down into the smallest bronchi, forming everywhere a complete lining to the tubes. There is no free fluid or froth in trachea or bronchi, as is constant in other forms of gassing. In the medium and small bronchi the membrane occludes the lumen, causing patches of atelectasis. These may be only 0.5 to 1 cm. in diameter, or may involve a whole lobe, depending upon the size of the occluded bronchi. Such atelectatic areas are dark reddish purple, depressed and nearly airless. Their relation to the plugged bronchi can be easily determined. No pleural effusion noted in animals.

No constant changes were found in other organs. Focal necroses were noted in the liver. These appeared on the surface and on cut section as gray spots, size of pinhead or smaller with a central red point. In three animals punctate hemorrhages were found in the adrenal cortex. In about half of the animals swelling and hyperemia of the mucosa of stomach and duodenum was noted; in one case circumscribed hemorrhagic ulcerations were found near the pylorus. No positive relationship of these findings to the gassing was demonstrated.

Haldane[13] noted the pathologic changes in two cats, one exposed two hours and killed four days afterwards; and the other exposed one and one-half hours and killed nine days after. The first cat showed desquamation of tracheal mucosa and the formation of a plug of leucocytes and red blood cells. Similar lesions, but more severe, were found in the bronchial tree; and the lungs showed leucocyte exudation into alveoli; edema and desquamation of alveolar epithelium, and abscesses. The second cat showed thickening and collapse of the alveolar walls,

congestion of the pulmonary vessels, desquamation of nasal epithelium, and inflammation of the skin of the ears.

McNee[14] gives autopsy summary of fatal cases. The points noted were acute inflammation of air passages, desquamation of mucous membrane, and formation of false membrane. To this, infection is added in the form of acute purulent bronchitis with atelectasis and bronchopneumonia. Acute emphysema may be present also. Superficial burns are a prominent feature. Acute hemorrhagic nephritis and edema of lower limbs were noted. In two cases degenera-

Fig. 1.—One hour after application of mustard gas. Stage of erythema.

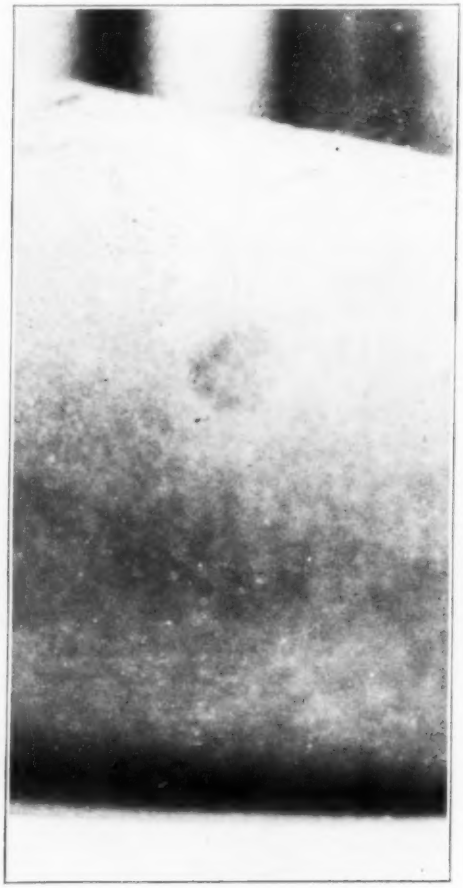

Fig. 2.—Three hours after application. Widening and deepening of the erythematous zone with well-marked edema. Development of secondary paler areola.

tive changes in the central portion of liver lobules and minute hemorrhages in brain were noted.

McLeod[15] from seven autopsies on men killed in action by the gas, concludes that the danger to life from secondary infection is greater than the toxic action of the gas itself.

Dunn[16] from four cases states that the chief lesions are: 1, severe and last-

ing damage to bronchi and bronchioles; 2, persistence of edema; 3, severe infection of respiratory tract, bronchopneumonia, and in two cases hemorrhagic exudations.

Summary.—The material given above represents the entire pathologic knowledge of mustard gassing available in the literature at the present time. As is readily seen, this pathology is very superficial and incomplete, being made up almost entirely of rather loose superficial gross descriptions. No detailed gross pathologic observations exist at present; and there is practically no microscopic

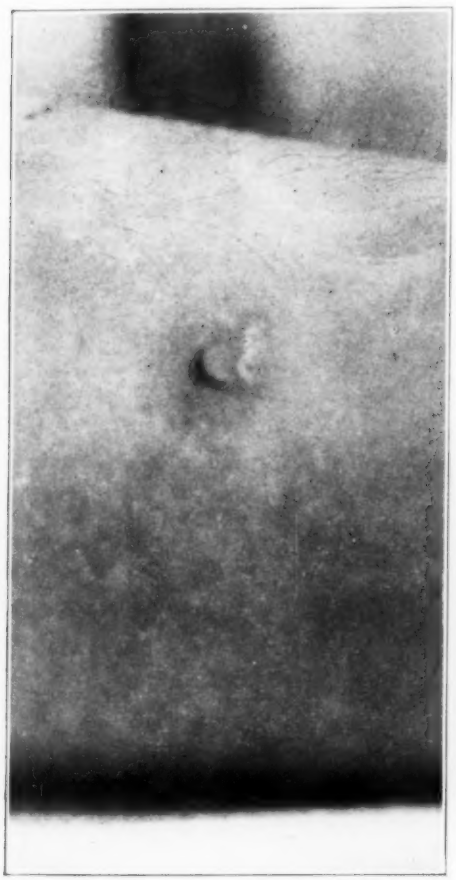

Fig. 3.—Eighteen hours after application. Formation of vesicle on an erythematous base.

Fig. 4.—Twenty-two hours after application. Vesicle 4 mm. high, tense, fluid content slightly opalescent. Floor of vesicle appears yellowish white, necrotic.

pathology. If a histologic study of the lesions produced by mustard gas exists in the literature, we have not been able to find it. For this reason, as soon as we heard of the new gas last summer, our laboratory made efforts to secure the substance for the purpose of experimental pathologic work with the view of determining the exact nature of the tissue lesions. Through the kindness of Professor Moses Gomberg, we were furnished with an ample supply of pure mustard gas, and more recently through the courtesy of Doctor Wm. Hale and

the Dow Chemical Company of Midland, Michigan, with an impure form of the gas. The great majority of our experiments have been carried out with the pure dichlorethylsulphide furnished by Doctor Gomberg. This is a clear, colorless, heavy, oily liquid having a boiling point of 217° C. and possessing a faint mustard odor.

The present investigation concerns itself solely with the study of the gross and microscopic pathology of the cutaneous lesions, produced in man and animals by the direct application of the liquid. The human material was obtained from

Fig. 5.—Forty-six hours after application. Collapse of vesicle with nearly complete absorption of fluid. Epidermis in fine wrinkles and folds, and of a yellowish brown tint.

Fig. 6.—Seventy-two hours after application. Beginning eschar formation. Central area bright yellowish brown in color, surrounded by a whitish zone 1-2 mm. in width.

auto-application, amputation material with consent of the patient, and accidental chemical laboratory lesions. Through auto-application we have been able to study a complete series of lesions through all stages from inception to healing.

ACTION OF DICHLORETHYLSULPHIDE ON HUMAN SKIN

The dichlorethylsulphide was applied by means of a capillary pipette in uniform droplets estimated to be about .002 c.c. When applied to the skin this drop

at once spreads out over an area 3 to 4 mm. in diameter and is completely volatilized, or at least disappears, in one to two minutes, according to atmospheric conditions.

Following is a protocol of the most important stages in the development of the skin lesion produced in this manner. We have found that the rate of production of the lesion and the intensity of the reaction vary over a considerable range in different individuals, notably in a Charcot-joint leg and in a case of malignant disease the development of the vesicle was delayed.

Fig. 7.—Four days after application. Beginning sloughing. Loss of necrotic wrinkled epidermis. Persistence of white zone and zone of erythema.

Fig. 8.—Nine days after application. Beginning separation of necrotic base from the peripheral white zone. More marked erythematous zone.

Auto-application to skin of normal individual. March 12, 1918, 2:30 P.M. Standard droplet applied to flexor surface of left forearm. In one minute the liquid completely disappeared giving off a strong mustard odor. There were no subjective symptoms. In about ten minutes there appeared a delicate silvery gray sheen over the surface of the area of application. This was soon followed by a faint flush which gradually deepened and spread until it was about 7 mm. across. Photograph in Fig. 1 was taken one hour after application. At this stage the erythema was influenced by changes of temperature, etc., alternately paling and reddening. Whenever the area became somewhat paler the superficial silvery luster was visible. During the second and third hour a well-marked edema appeared and the erythematous zone became wider and more deeply colored.

Same day, 5:30 P.M. At this time the lesion measured 15 mm. in diameter. It was slightly elevated and had a marginal zone somewhat deeper red than the central portion. Outside of the red zone there was a very faint, barely perceptible zone showing less color than the remainder of the skin. (Fig. 2, photograph three hours after application.) In the next several hours there was no change.

March 13, 1918, 6:30 A.M. Sixteen hours after the application a vesicle began to form. At 8:30 A.M., eighteen hours after the application this was at its height. (Fig. 3.) At this time the lesion measured 25 mm. in diameter. It presented an erythematous base, slightly elevated, and fading gradually into the surrounding skin. Upon the summit of this erythematous base, there rose a tense vesicle 6x9 mm. in area and 3 mm. high. This was filled with a clear pale yellow fluid. Up to this time there had been no subjective

Fig. 9.—Eighteen days after application. Raising and separation of the yellowish brown crust. Beginning fading of the erythematous area leaving a pigmented border.

Fig. 10.—Nineteen days after application. Base of lesion after complete separation of the heavy crust of the eschar. Granulation tissue base.

symptoms, but with the formation of the vesicle there was slight smarting, increased by pressure.

Same day, 12:30 P.M. Twenty-two hours after application. (Fig. 4.) At this time the vesicle covered no greater area but was 4 mm. high and still very tense. Its fluid content had become slightly cloudy or opalescent. With a hand lens the base of vesicle can be seen through the fluid content and appears yellowish white, opaque and necrotic. Around the border of the inflamed base of the vesicle there is a definite secondary areola.

March 14, 1918, 12:30 P.M. Forty-six hours after application. (Fig. 5.) By this time there was nearly complete absorption of the fluid from the vesicle. The epidermal

covering of the vesicle is thrown into fine wrinkles and folds and has taken on a yellowish brown tint. The zone immediately around the base of the vesicle is now pale pinkish white and about 1 mm. in width. Outside of this the flushed areola persists. The total width of the lesion is now but 17 mm.

March 15, 1918, 2:30 P.M. Seventy-two hours after application. (Fig. 6.) The central portion of the lesion measures 8x4 mm. and is of a bright yellowish brown color. Around this is a white zone 1 to 2 mm. in width. Outside of this is a zone of erythema most marked about the base of the collapsed vesicle and fading peripherally. The total width of the lesion is now 18 mm.

During the night of March 15 to March 16 the delicate wrinkled epidermis was rubbed off, leaving an excavated area with a grayish yellow-white moist base. The excavated area measured 6x4 mm. The border was somewhat irregular and slightly over-

Fig. 11.—Forty-nine days after application. Scar with brown pigmented areola. Slight puckering of scar.

hanging. The white zone, about 1 mm. in width, still persists at the border and the erythematous zone outside of this is about the same width as before. The base became slightly glossy upon drying. Fig. 7 was taken at 5:30 P.M., March 16, 1918.

March 17, 1918, 9:00 P.M. Total excavated area somewhat diminished. Border smoother. Floor not quite so deep. Total width of lesion is now 15 mm.

March 21, 1918, 4:00 P.M. A brownish crust representing the necrotic base is beginning to loosen at the edges where it is white and slightly desquamating. About this is a marked erythematous areola, 4 mm. wide. (Fig. 8.)

March 22, 1918, 9:00 A.M. During the night there was marked itching, the only pro-

nounced subjective symptom so far noted. The crust loosened entirely and came off. Beneath the crust there was a small amount of thin purulent fluid. The erythematous zone is less marked. The base of the excavated area is again dry and covered with a yellowish brown crust. The margin of the excavation is whitish with edges slightly puckered. The central portion of the lesion measures 5x9 mm.

March 30, 1918. Since March 22, the area of excavation has gradually become covered with a yellowish brown crust which has now become elevated nearly 2 mm. above the surrounding surface. The zone of erythema is fading, leaving a yellowish-brown pigmentation. The inner portion of this zone is shiny and somewhat puckered and there are a few minute desquamating scales at the border of the dense crust. The entire width of the lesion including the zone of pigmentation is 3 cm., the reddened, somewhat shiny

Fig. 12.—Typical "mustard gas" vesicle. About twenty-two hours after application. Photograph somewhat enlarged to show details of the vesicle.

zone, is 2 to 3 mm. in width, the scaly desquamation about 1 mm., and the elevated crust 4x7 mm. (Fig. 9.)

April 1, 1918. The dark crust or scab became loose and came away leaving a white dry slightly granular area nearly flush with the surface of the skin. This measured 4x8 mm. (Fig. 10.)

May 1, 1918. Healing is now nearly complete, the lesion consisting of a thin scar, pinkish white in the central portion and whiter, more opaque, at the margin, with very slight puckering. Around this is a brown pigmented areola. The whole area, however, is redder than normal skin. (Fig. 11.)

MICROSCOPIC APPEARANCES

The changes in human skin were studied microscopically from one-half hour up to thirty-six hours, including the development of the vesicle and beginning eschar formation. As the lesions at one-half hour, eighteen hours, and thirty-six hours represent three distinct stages, these will be described in detail.

Lesion One-half Hour after Application

Epidermis.—The horny layer is relatively thicker than normal and split up into flat scales and layers, loosening readily from the stratum lucidum. The stratum lucidum has a slight brownish color. The granular layer is flattened; the cells drawn out parallel to the surface; the nuclei are pyknotic. The stratum germinativum is markedly shrunken, in many places only one-third to one-half

Fig. 13.—Mustard-gas burns of both hands, six days after exposure, in a chemical laboratory assistant.

as wide as normal, its nuclei pyknotic and the cytoplasm shrunken about the nuclei. Occasional vacuoles are found in the lowest layers, but the most marked change is the shrunken appearance of the whole epidermis, both cytoplasm and nuclei. At the border of the lesion the epidermis passes gradually into the normal condition.

Papillary Layer of Corium.—In the central part of the lesion, the capillaries are contracted and contain but little blood. In scattered capillaries the red blood cells are agglutinated and stain with eosin as bright red hyaline masses. Such agglutination thrombi, however, are not a common feature of the picture and in the larger vessels thrombosis does not occur. The endothelium of the capillaries of the papillary layer shows marked pyknosis, karyorrhexis and disintegration of the nuclear chromatin. Chromatin dust is found around many of these capil-

laries and also many of the connective tissue cells of the upper portion of the papillary layer show marked karyorrhexis. The cytoplasm of many of the endothelial cells of the capillaries is vacuolated, showing hydropic degeneration or edema. About these capillaries there is a clear space due to a perivascular edema. Around some capillaries this is very marked. Many of the capillaries show diapedesis of leucocytes along their course (Fig. 15), but in the central

Fig. 14.—Scar on back of hand six months after accidental burn with a dilute solution of dichlorethylsulphide dropped upon back of hand and immediately washed away. Chemist. Burn exhibited stages of vesicle and eschar formation similar to those described in the above protocol, followed by very slow healing.

part of the lesion there is practically no hemorrhage and the vessels are conspicuous for their contraction and anemia.

Corium Proper.—The vessels running through the corium show similar changes in their endothelium but there is little leucocytosis or white cell migration. The larger vessels contain more blood. No thromboses or hemorrhages are present. The lymphatics are dilated and the nerve trunks show karyorrhectic nuclei and edema.

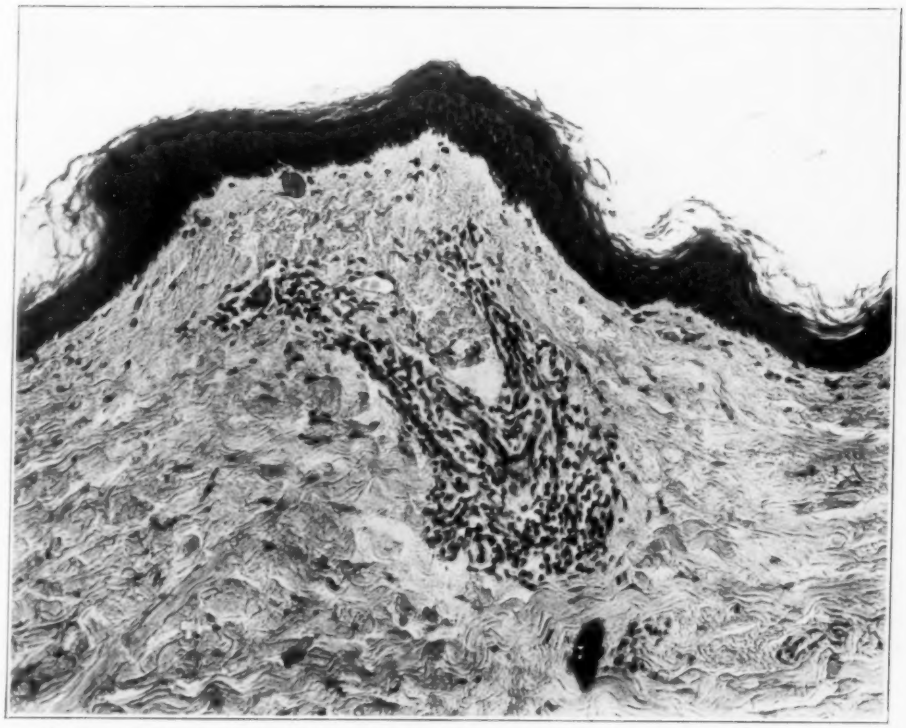

Fig. 15.—Human skin one-half hour after application of mustard gas. Shrinking and pyknosis of epidermis with desquamation of horny layer. Capillary changes as described in the text, including well-marked migration of leucocytes.

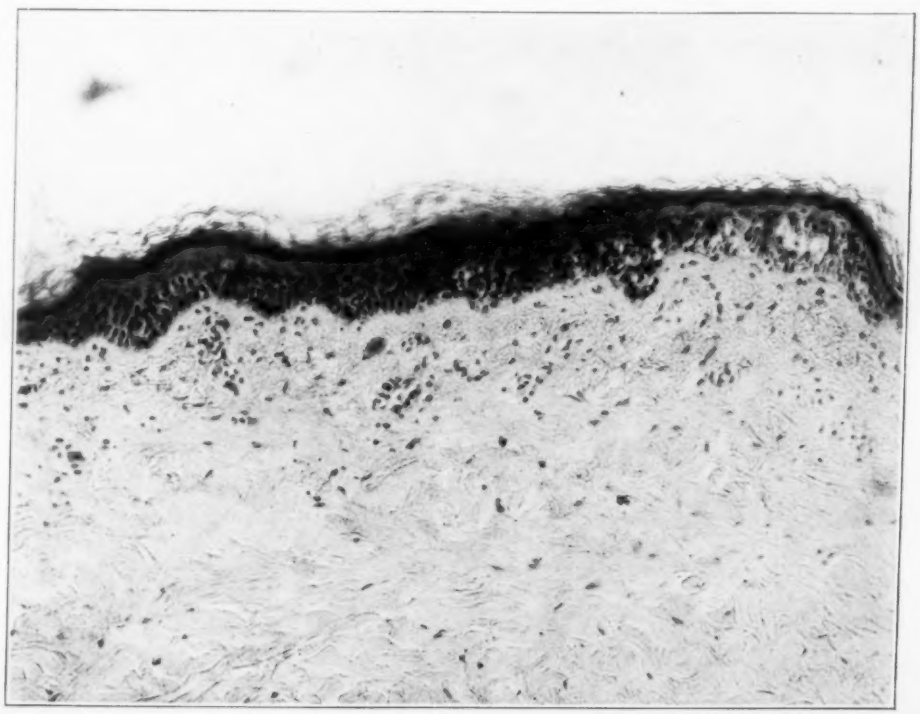

Fig. 16.—Human skin eighteen hours after application. Transition between slightly damaged epithelium and epithelium showing hydropic degeneration. Early blister formation.

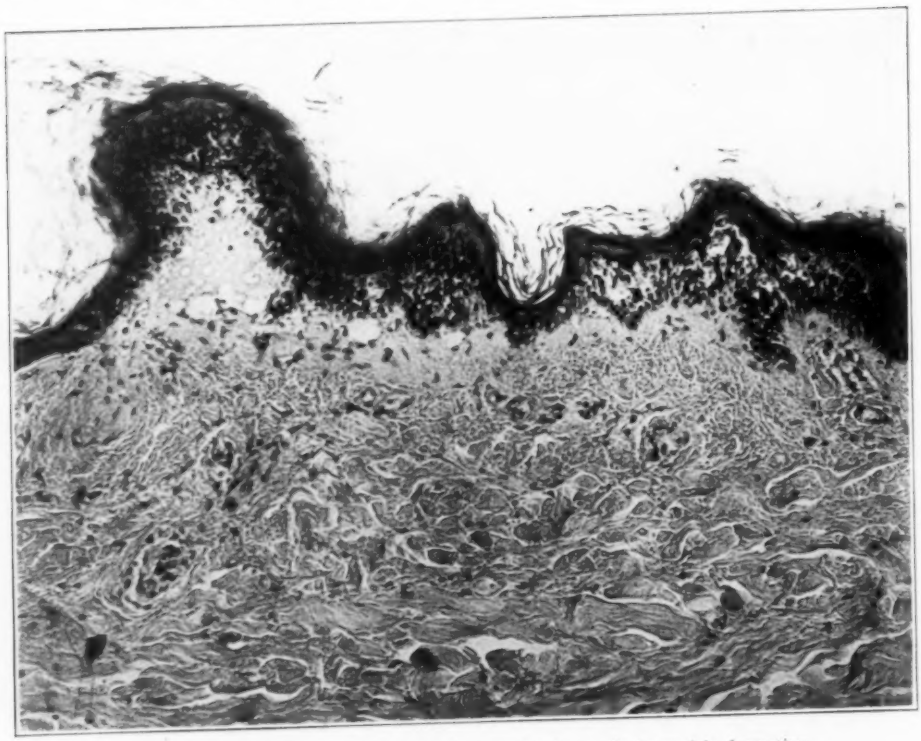

Fig. 17.—Human skin eighteen hours after application. Early vesicle formation.

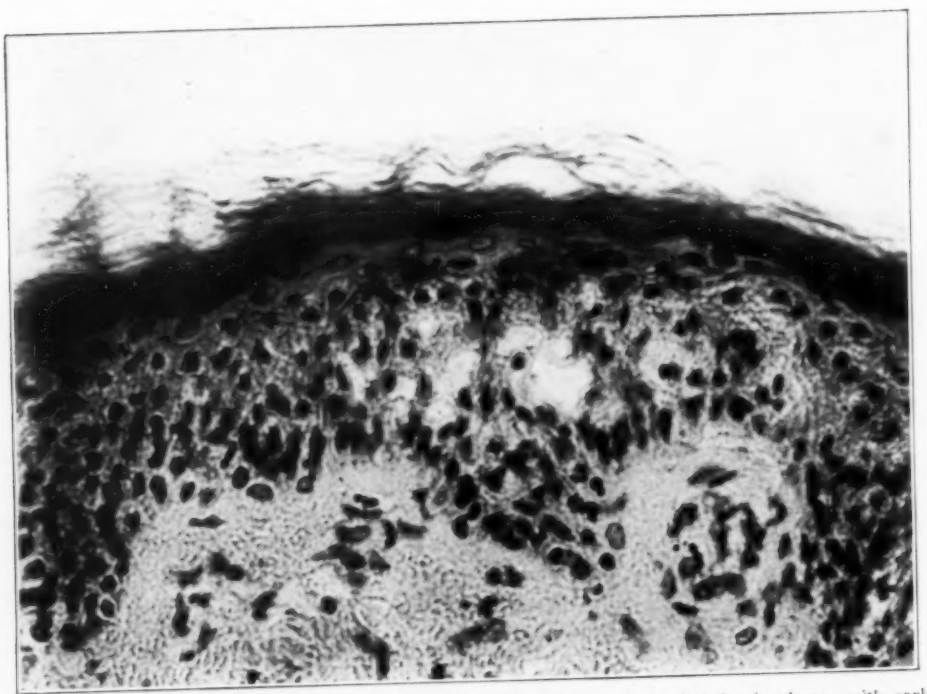

Fig. 18.—Human skin eighteen hours after application. High power view of hydropic change with early vesicle formation.

Hair Follicles.—Along the hair follicles the squamous epithelium shows changes similar to those of the surface and the capillaries about the hair follicles also show changes similar to those described above. In the neighborhood of the hair follicles the corium is affected more deeply than elsewhere, showing a distinct penetration through the hair follicles.

Sweat Glands.—The epithelium of the sweat glands shows no apparent changes, although the vessels about them show changes similar to those described above.

Sebaceous Glands.—Shrinking and pyknosis of cells similar to that seen on the surface.

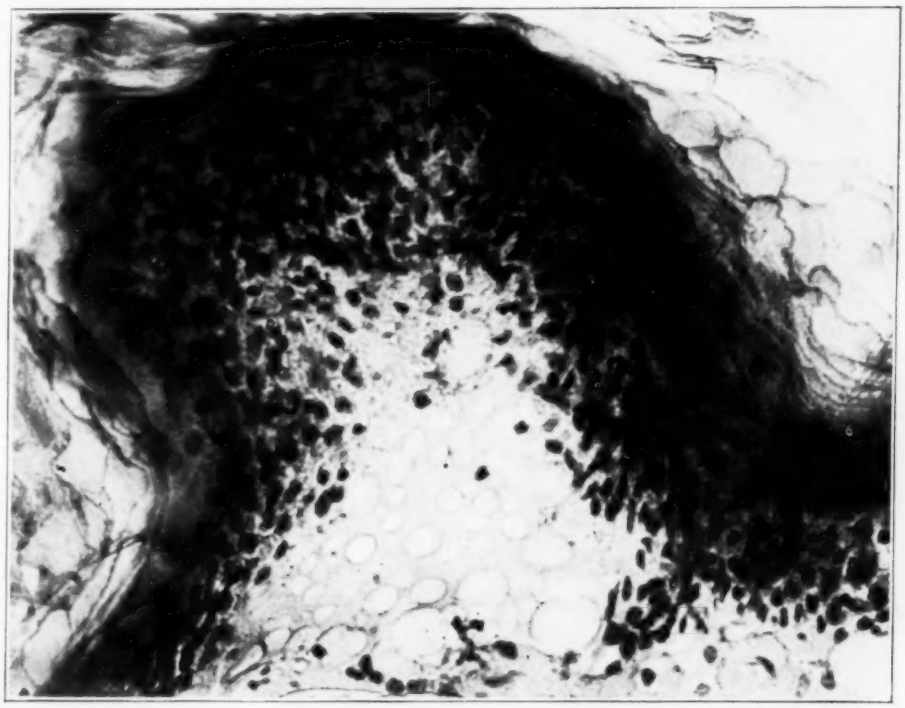

Fig. 19.—Human skin eighteen hours after application. High power view of small vesicle. Separation of epidermis from papillary layer.

In the transition border there are small hemorrhages by diapedesis.

Lesion Eighteen Hours after Application. (*Figs.* 16, 17, 18, *and* 19.)

Epidermis.—In the central part of the lesion there is a marked liquefaction and hydropic change in the cytoplasm of the epithelium. This varies greatly in degree. The horny layer is in part desquamated and loosened, the stratum lucidum is widened and more dense than normal and stains brownish red. Over many of the papillæ small vesicles have already formed, the majority of the epithelial cells having undergone liquefaction. In some places the epidermis is lifted from the papillæ by the collection of fluid beneath it. The liquefaction of the cell cytoplasm extends deep down into the hair follicles and into the sebaceous

Fig. 20.—Human skin thirty-six hours after application. Vesicle formation in epidermis and leucocyte infiltration of papillæ.

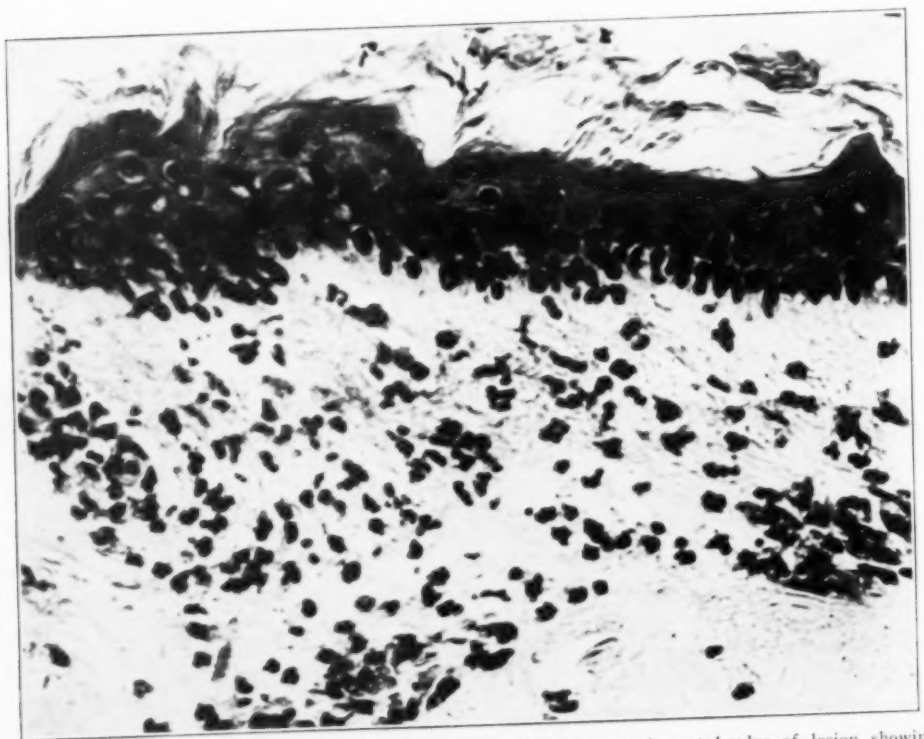

Fig. 21.—Human skin thirty-six hours after application. High power view at border of lesion showing changes in epidermis and leucocyte infiltration and edema of papillary layer.

glands. The stratum germinativum has lost its continuity in many places and the cells are completely necrotic.

Papillary Layer of the Corium.—The connective tissue is edematous, stains bluish, and contains many degenerating nuclei. There is an increase in the number of wandering cells and many of these show karyorrhexis. Around all the capillaries there is a zone of edema and small-celled infiltration. Small hemorrhages by diapedesis are scattered through the papillary layer and upper portion of corium, particularly around the hair follicles.

Corium Proper.—The blood vessels contain more blood than in the earlier lesion, particularly the deeper ones. The larger ones show a marked congestion and the lymphatics are dilated with a lymph rich in albumin. Around the hair follicles the edema, liquefactive changes, and hemorrhages are more marked than elsewhere. The sweat glands show a marked edema of the interstitial connective tissue, congestion of the capillaries, leucocyte infiltration and small hemorrhages by diapedesis. Some of the glands show a marked necrosis of the epithelium but these changes vary greatly in degree.

Subcutaneous Tissues.—The vessels are congested, lymphatics dilated and there is edema of the adipose tissue. The vascular changes extend along the smaller capillaries even into the subcutaneous tissue.

Lesion Thirty-six Hours after Application (Figs. 20 and 21)

Epidermis.—Horny layer more compact but ragged, in many places infiltrated with leucocytes. Epidermis nearly completely necrosed, in some places lifted from the papillary layer. The remaining nuclei are markedly pyknotic or fragmented. In many areas only the lowest layer of nuclei persists. There are also collections of fluid between the horny layer and the portions of rete remaining.

Papillary Layer.—The papillary layer shows marked edema, the capillaries are congested and there are many hemorrhages by diapedesis. The entire papillary layer is infiltrated with leucocytes, many of which show karyorrhexis.

Corium.—There is a leucocyte infiltration throughout the entire corium but less marked than in the papillary layer. It is most marked around the hair follicles and around the sebaceous glands and sweat glands. The congestion and edema are also most marked around these structures. Some of the smaller vessels show marked necrosis of the wall with leucocyte infiltration and diapedesis. Scattered areas of edema and small celled infiltration extend even into the subcutaneous tissue where the vessels are markedly congested.

Later Stages in Human Skin

Inasmuch as the later stages in human skin parallel those in the lower animals and as the specific differences between the action of mustard gas on human skin and on the skin of the rabbit, guinea pig and cat exist only in the early stages, it seems necessary here to omit a more detailed description of these changes and to summarize them as follows:

1. About forty to fifty hours after application collapse of vesicles and progressive necrosis.

2. About seventy-two hours after application progressive necrosis and beginning eschar formation.

3. Four to six days after application, necrosis complete, beginning separation of slough. Edema and hyperemia persistent.

4. By the nineteenth day, complete separation of slough. Slow healing and scar formation.

5. For an indefinite period, congestion and pigmentation.

All of these descriptions applied to the effect of a standard drop of pure mustard gas in the absence of infection. The course will naturally vary with the concentration, amount, time, etc.

PREVENTION OF THE LESION

A preliminary report is made here of methods tried out to prevent or abort the lesion after the application of mustard gas. We still regard this work as in the experimental stage and the statements made here only tentative. In some of the English and French reports great emphasis is laid on the fact that water greatly increases the intensity of the lesion. Our experience shows this to be unfounded. If the application of mustard gas to the skin is followed by thorough washing within a few minutes, the resulting lesion is far less intense though usually spread over a larger area. If this can be done before the expiration of two minutes, no necrosis and no vesicle formation results and there is only a hyperemia and a more or less transient edema. Washing immediately with tincture of green soap absolutely prevents the development of a lesion and if this is done two minutes after application, just as the droplet has completely volatilized, only a slight but persistent hyperemia results. This method of prevention, while it might not be applicable under the conditions of warfare, would apply to prevention in factories, chemical laboratories, and munition depots. Bleaching powder, sulphur chloride, and sodium bicarbonate are also of value but from our present results we would recommend immediate washing with tincture of green soap.

ANIMAL EXPERIMENTS

The rabbit, guinea pig, and cat were employed for these experiments. The skin of the belly was shaved and standard drops were applied after the irritation from shaving had subsided. The character of the tissue lesion and the reaction in these animals, proved, in the early stages, to be essentially different from the lesions in human skin. For the purpose of brevity and conciseness the protocols are condensed as below.

Rabbit.—Within two hours after application of the standard drop there develops a very marked edema, much larger than the area touched by the drop of mustard gas. This edema is subcutaneous, appearing as a definite tumor mass, rather sharply circumscribed, over which the cuticle can be moved. The surface of the area appears gray and cloudy, the skin losing its normal translucency and appearing as if cooked. In some cases the blanching appears to extend into the deeper portion of the skin. About this gray area there is but slight hyperemia.

By the third day after the application, the epidermis over the area undergoes complete necrosis and there is formed a slough without any vesicle formation. Vesicles were never observed in the rabbit. This slough is held on apparently by the hairs. It gradually is elevated, separates, and contracts, and may not be cast off for three or four weeks. When shed, the lesion below is practically healed. The most striking thing is the marked edema at the beginning, the per-

Fig. 22.—Rabbit. Application of mustard gas at 11:30 A.M. Droplet used was slightly larger than the standard. Marked subcutaneous edema as seen at 4:00 P.M. on the same day.

sistence of this edema without vesicle formation, and the slow healing in the absence of infection.

Various protective experiments were tried out, a number of substances being used to prevent the lesion or lessen its severity—washing with water, soap and water, lead acetate, lead acetate and silver nitrate, zinc oxide ointment, zinc oxide paste, bleaching powder, sodium sulphide, tincture of green soap, potassium permanganate. The application of most of these substances five minutes

after the application of the mustard gas lessens the edema and renders the lesion more diffuse but does not prevent necrosis. The use of potassium permanganate resulted in even greater edema than the untreated control while strong sodium sulphide solution was found to be disadvantageous because of the necrosis produced. (Figs. 22, 23, and 24.)

Fig. 23.—Rabbit. Skin of belly shows results of four applications of standard drops of mustard gas. Above two areas, of typical edema, the one on the rabbit's right untreated, the one on the left washed off in five minutes by water. The latter is more diffuse, larger in area, but less intense. Below on the rabbit's right, an area washed after five minutes with soap containing an excess of free alkali. This area shows the least reaction. On the lower left is an area treated after five minutes with potassium permanganate. The reaction here is the most marked.

Guinea Pig.—Practically identical results were obtained in the guinea pig with the standard drop of pure mustard gas; namely, within a few hours marked subcutaneous edema followed in a few hours by necrosis of epidermis and papillary layer without vesicle formation and exhibiting very slow healing.

Cat.—The same results were obtained as for rabbits and guinea pigs. Sub-

cutaneous edema without vesicle formation, followed by necrosis and slow healing.

MICROSCOPIC PATHOLOGY OF ANIMAL LESIONS

The changes observed in the development of the lesions in the rabbit, guinea pig, and cat are essentially the same and are summarized here as follows:

1. *Stage of Marked Edema.*—The most striking feature of this stage is the

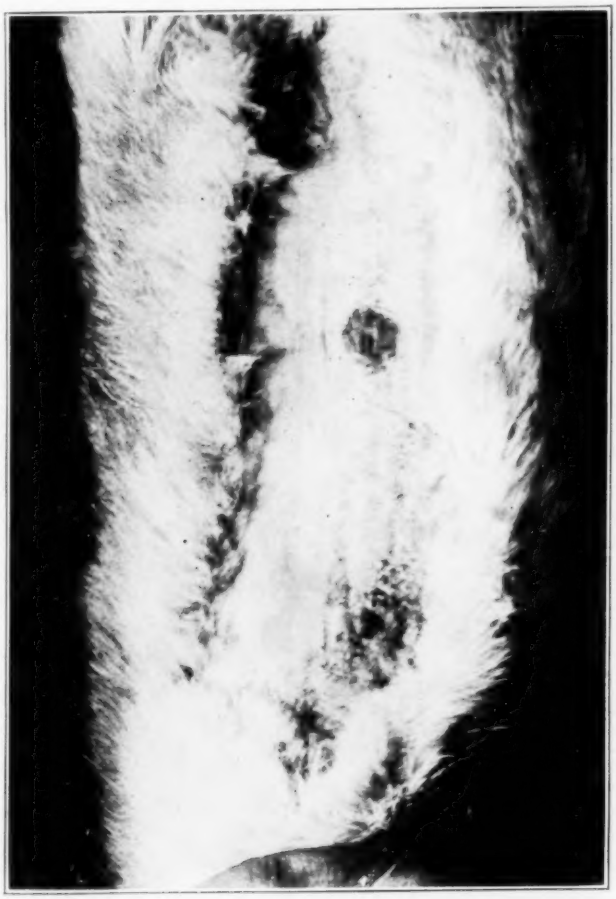

Fig. 24.—Rabbit. Two areas of mustard-gas application. Advanced eschar formation.

intense edema which is sharply localized to the subcutaneous tissue and fascia, but extends into the muscle through the abdominal wall. The connective tissue fibrillæ are widely separated and the tissue spaces are filled with a heavy albuminous precipitate staining deep pink with eosin. The muscle fibers of the abdominal wall are separated, and even in two hours there is a leucocytic infiltration into the muscle. The edema extends 0.5 to 1 cm. below the epidermis. The epidermis is shrunken, cells pyknotic and in the central portion of the lesion completely necrotic. In the upper layer of the corium numerous degenerating nuclei are seen. The blood vessels show degeneration of the endothelium with small

hemorrhages by diapedesis and leucocyte migration. Around each vessel there is an area of edema. There is, however, no vesicle formation in or beneath the epidermis as in the human cases. The changes are more uniformly diffuse in the animal than in the human skin and the depth of penetration greater. The

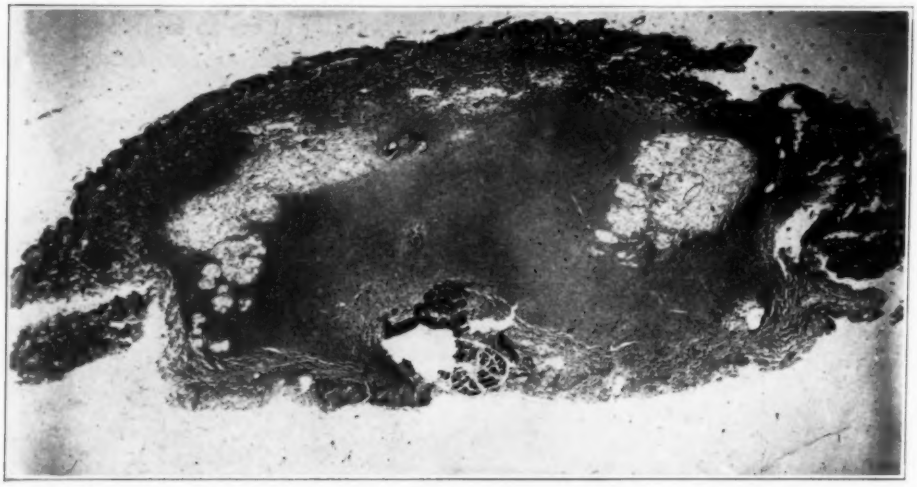

Fig. 25.—Rabbit. Low power view of mustard-gas lesion in rabbit two hours after application. Extreme subcutaneous edema. Epidermis but slightly changed.

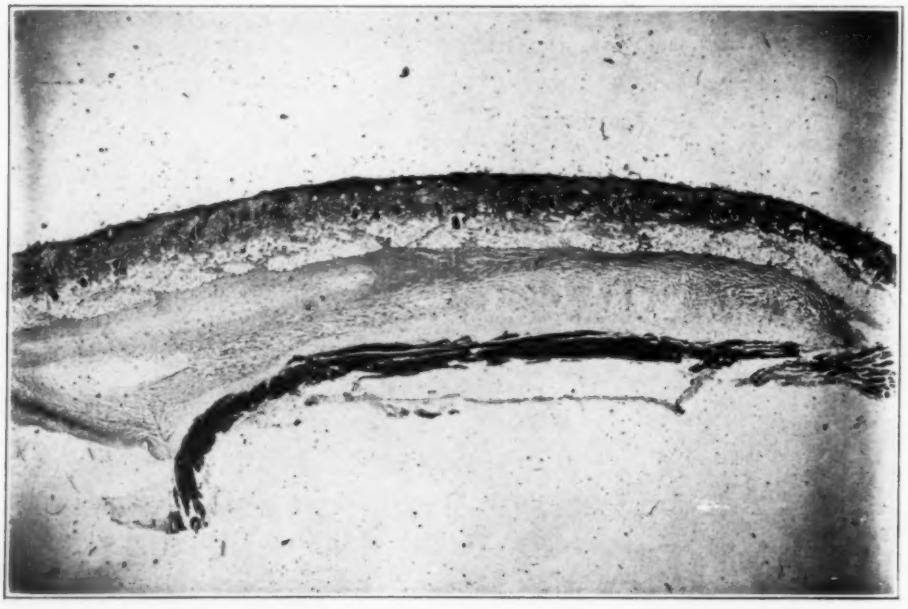

Fig. 26.—Guinea pig. Low power view of mustard-gas lesion five and one-half hours after application. Extreme subcutaneous edema. Epidermis necrosed in center of lesion.

localized penetration along the hair follicles, so prominent in the human skin, does not show in the animal skin, the greater number of hair follicles permitting a more uniform access of the liquid. There are no thromboses in the damaged

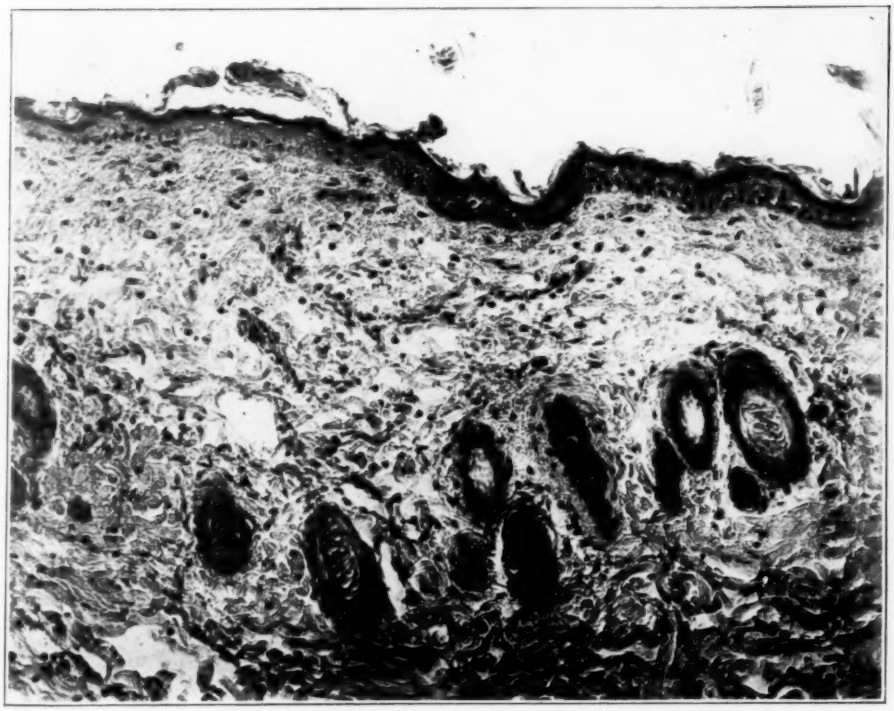

Fig. 27.—Rabbit. Border of lesion two hours after application. To the right of the middle the epidermis is still living, to the left nearly completely necrosed, necrosis extending into the upper portion of the corium. Early edema.

Fig. 28.—Rabbit. Two hours after application. Changes in epidermis and corium. Marked vascular change with beginning migration of leucocytes. Small hemorrhages by diapedesis. Early edema.

area. The hemorrhages are relatively small and, as in the human skin, the vessels in the immediate lesion are contracted and anemic. Around the borders of the lesion they show marked congestion.

2. *Stage of Necrosis.*—The necrosis of the epidermis and of the underlying tissues steadily becomes more prominent because of the loss of nuclei in the epidermis and upper part of the corium of the central part of the lesion. By the fifth and sixth day after the application of the mustard gas the edema has subsided to a marked degree and the central part of the lesion may be entirely without nuclei as far as the lower portion of the corium. It is bloodless, rather dry,

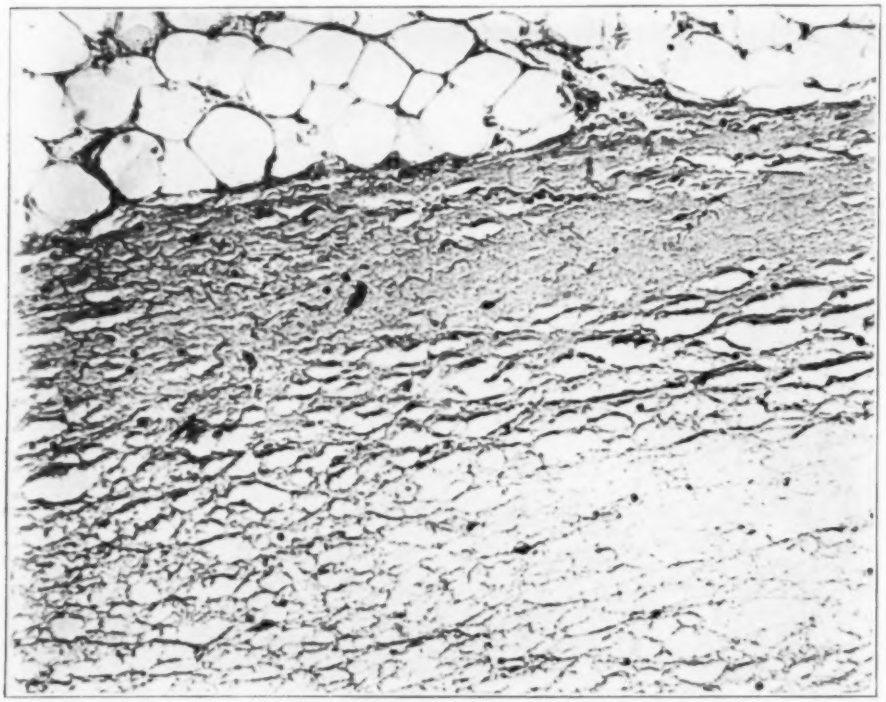

Fig. 29.—Guinea pig five and one-half hours after application of mustard gas to skin of abdomen. Deep subcutaneous edema.

and there is but little leucocyte infiltration. Surrounding this is a narrow zone of less marked necrosis and degeneration. This gradually becomes hyperemic and small hemorrhages by diapedesis may take place from the damaged vessels. In this area there is a more marked infiltration of leucocytes but this rarely becomes diffuse, the leucocytes remaining collected in the neighborhood of the vessels. Outside of this zone the tissues are hyperemic, somewhat edematous and show an increased number of wandering cells for some distance.

3. *Stage of Eschar Formation.*—There gradually begins a separation of the completely necrotic tissue from the living. This eschar consists of the dead epidermis and upper part of the corium, sometimes as far down as the lower borders of the hair follicles. This drys, contracts, becomes leathery, but is held in position by the hairs. There now develops in the neighboring living tissue a productive inflammation. The shrinking of the necrosed tissue and the demarcation,

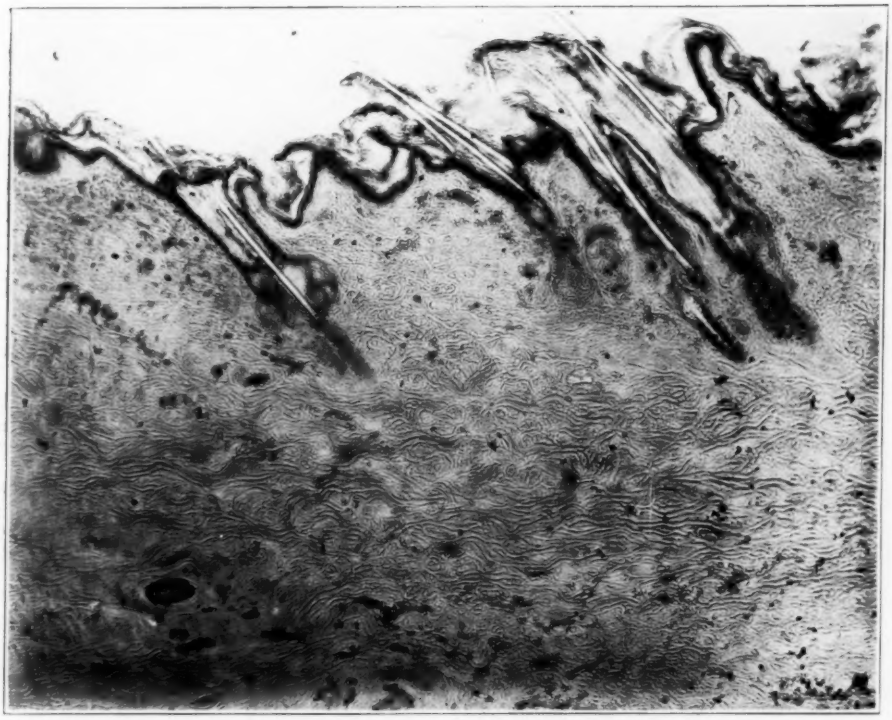

Fig. 30.—Rabbit. Six days after application. Treatment with zinc oxide paste five minutes after use of mustard gas. Center of lesion. Complete necrosis of epidermis, hair follicles and upper portion of corium, extending even to the sweat glands. No reaction.

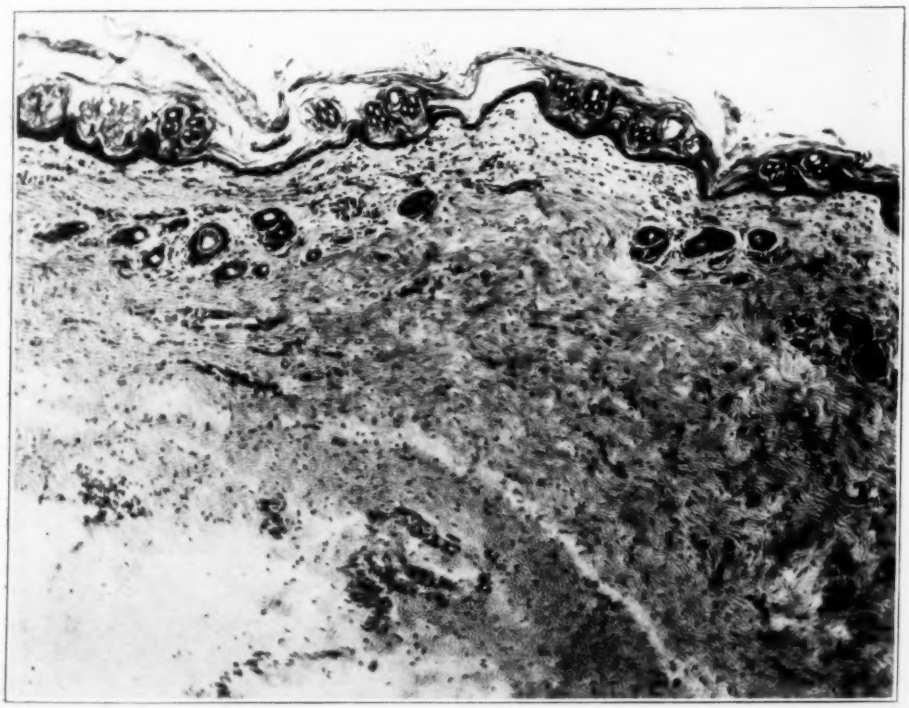

Fig. 31.—Rabbit. Six days after application. Treatment with zinc oxide ointments five minutes after application of mustard gas. There was no edema stage. Epidermis is dead and there is a moderate inflammatory reaction in the corium. Reaction much less intense than in control.

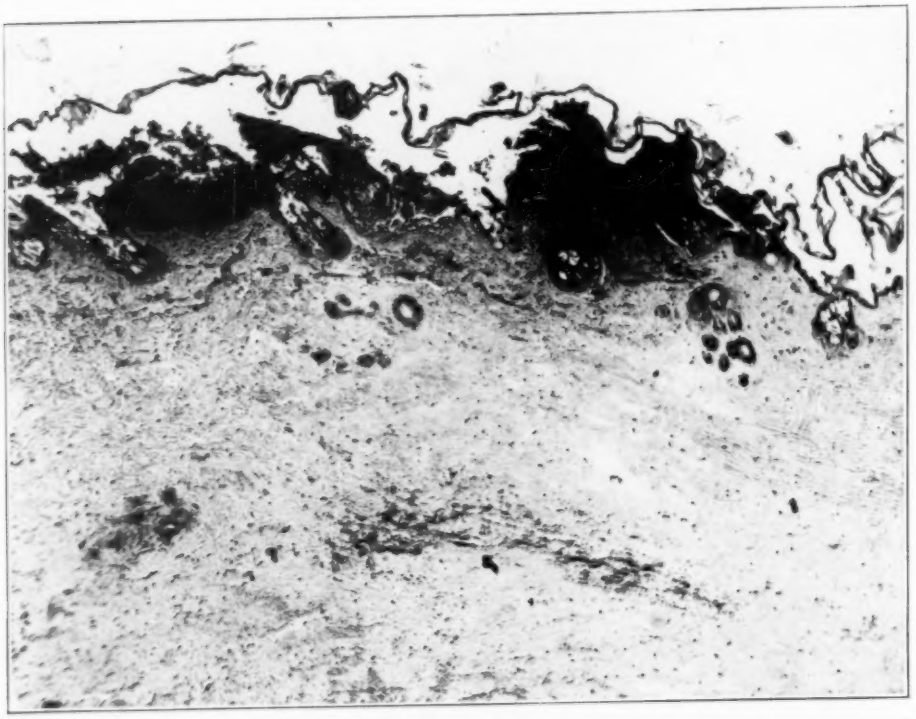

Fig. 32.—Rabbit. Six days after application. Treatment after five minutes with two per cent solution of silver nitrate and five per cent lead acetate. Primary edema was nearly completely controlled but necrosis six days after is marked, extending deep into the corium, with more rapid separation of the slough.

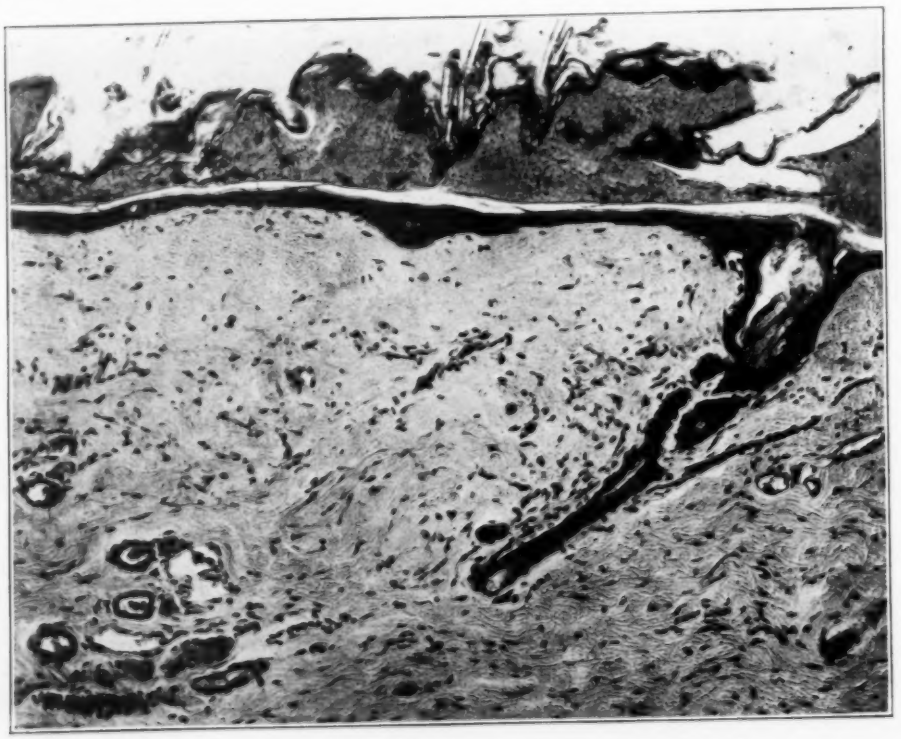

Fig. 33.—Rabbit. Periphery of same lesion as Fig. 32. Area of less damage.

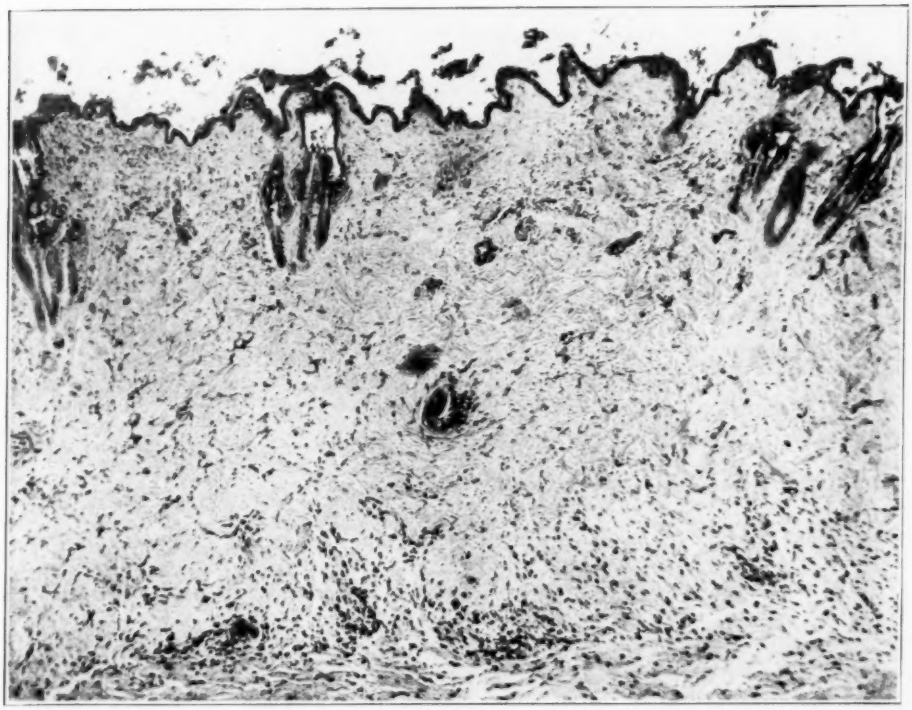

Fig. 34.—Rabbit. Six days after application, untreated. Border of lesion. Necrosis less marked. Beginning repair.

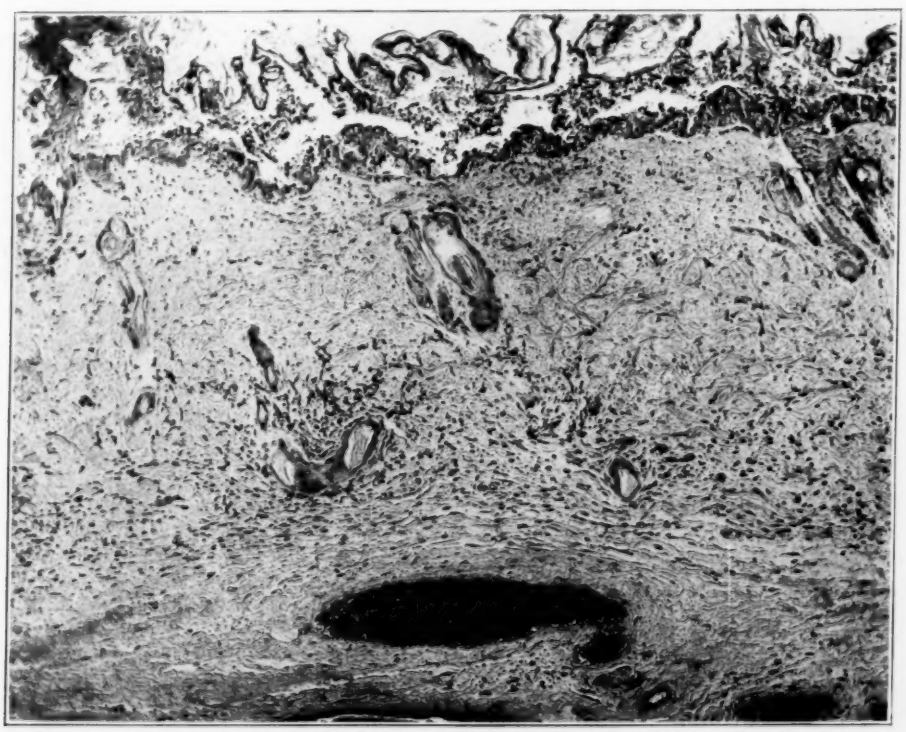

Fig. 35.—Rabbit. Six days after application, untreated. Intermediate zone. Separation of necrotic epidermis and papillary layer with infiltration of leucocytes into the necrotic tissue. Fibroblastic proliferation in lower part of dermis with regeneration of hair follicles. Intense congestion of subcutaneous vessels.

with the surrounding reparative inflammation, progress very slowly until there is a regeneration of the epithelium beneath the eschar. The latter remains adherent, usually until complete repair has taken place. The repair of the epidermis takes place chiefly from the cells remaining in the hair follicles.

(For the histologic changes in animal tissues see Figs. 25 to 36 inclusive.)

SUMMARY

1. Dichlorethylsulphide (mustard gas) is an escharotic, specific in its action upon the epidermis and tissues of corium, particularly upon the endothelium of the vessels.

2. The lesion is a chemical burn unlike that produced by heat, electricity,

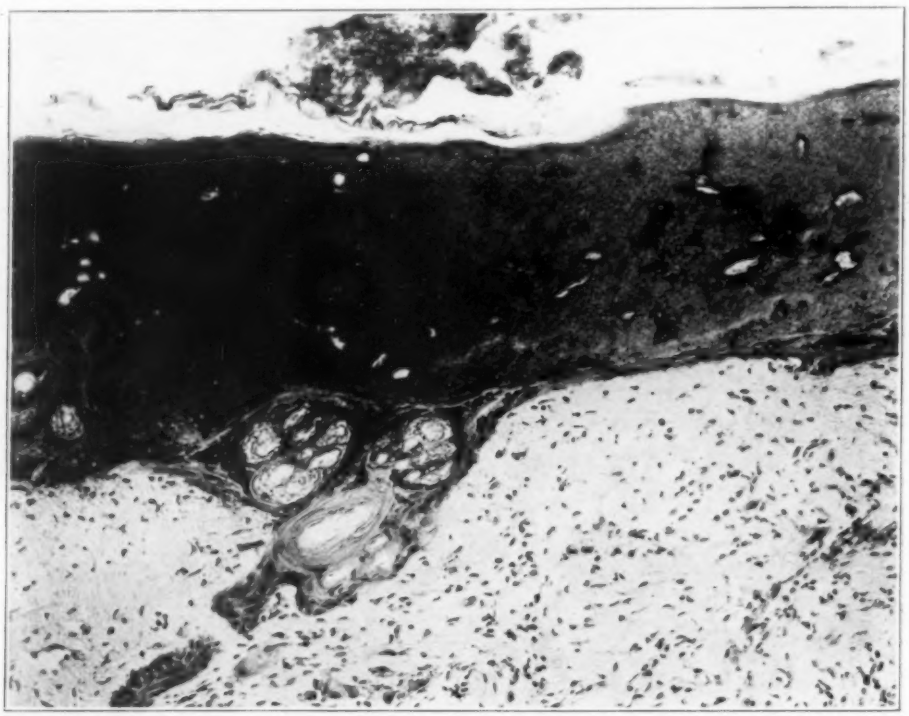

Fig. 36.—Rabbit. Six days after application, untreated. Adherent slough, representing the necrotic epidermis and upper portion of corium, involving the hair follicles.

or the ordinary corrosives such as sulphuric, nitric, and hydrochloric acids or strong alkalies. Of all these agents, the effects are most closely allied to those of hydrochloric acid, but are much greater in intensity. It differs from a heat burn in the absence of thrombosis, in the greater degree of fluid exudation, in the greater moistness of the affected area and in the fact that the necrosis as shown by the loss of nuclei requires hours, or even days, for its complete development. The coagulated, shrunken and cooked appearance of the tissues in heat burns is not apparent in the tissues of mustard gas burns.

3. The vessels in the affected area are severely damaged and collapsed and there is a local anemia in the earlier stages, with a marked fluid exudation and leucocyte migration. The process is nonhemorrhagic and nonthrombosing.

4. In man the necrosis of the epidermis is usually evident in two hours

through the hydropic change in the epithelium and early vesicle formation. There is no deep edema. It is confined to the epidermis and to the papillary layer in the early stages.

5. In animals the intense and deep edema is most striking and altogether different from that seen in man. Vesicle formation was not noted by us in animals.

6. The deep penetration of the smallest quantities applied to the surface is a most striking feature. There is an undoubted entrance through the hair follicles, sebaceous and sweat glands.

7. The slowly progressive character of the necrosis is a specific characteristic, the height of the necrosis being reached five to ten days after application. This may, in part, be explained by contraction and death of the vessels with resulting anemia in the affected area.

8. The painlessness of the lesion is also a marked characteristic. This may be explained by the edema and degeneration of the nerve endings in the affected portion.

9. In none of our animals was there any conjunctivitis or irritation of the respiratory tract produced by the cutaneous applications. We conclude that there is no evidence of metastasis from the local lesion as claimed by both Meyer and Haldane. We believe that the conjunctival and respiratory lesions are due alone to the direct action of mustard gas and when animals are protected from the vapor no lesions in these organs will result, no matter how severe the skin burn.

10. Contrary to the statements of certain English and French observers, the admixture of water does not increase the escharotic action, but if the oil is immediately washed away, the lesion is greatly reduced in intensity. Washing within two minutes with tincture of green soap may entirely prevent the lesion or result in only a slight hyperemia.

11. We believe that the lesions observed in the axilla, between the fingers and toes, around the genitals and between the thighs of men gassed in action are probably due to the greater moisture of these parts from perspiration and the resulting re-solution of the gas.

12. The slow healing is probably chiefly due to the vessel injury and the relatively slight leucocytic demarcating infiltration. In this respect the lesion is strikingly like an x-ray burn of the skin.

Our thanks are due to Professors Moses Gomberg and William Hale and the Dow Chemical Company for pure and impure dichlorethylsulphide, and to Dr. George Herrmann for aid in making human applications. Report on the pathology of the respiratory lesions and on the production of the conjunctivitis will be made later.

BIBLIOGRAPHY

[1]Meyer, Victor: Ber. d. deutsch. chem. Gesellsch., 1886, xix, No. 3, 3259.
[2]Meyer, Victor: Ber. d. deutsch. chem. Gesellsch., 1887, xx, No. 2, 1729.
[3]Giraud, Albert: Jour. de méd. et de chir. prat., Nov. 25, 1917, lxxxviii, 890-895.
[4]Teuliéres: Jour. de méd. de Bordeaux, Nov., 1917, No. 12, p. 247.
[5]Mandel and Gibson: Jour. Am. Med. Assn., Dec., 1917, lxix, p. 1970.
[6]Haldane: British War Reports.
[7]Kolls and Gilbert: British War Reports; British Medical Reports.
[8]Raper and Ball: Ibid.
[9]Marshall and Miller: Ibid.
[10]Kolls and Gilbert: Ibid.
[11]Ibid.
[12]Mackenzie: Ibid.
[13]Haldane: Ibid.
[14]McNee: Ibid.
[15]McLeod: Ibid.
[16]Dunn: Ibid.

A DISCUSSION OF THE LIPOIDS CONCERNED IN GROWTH WITH CLINICAL OBSERVATIONS ON THE ACTION OF TETHELIN*

By E. L. Barney. M.S., M.D., San Francisco, Cal.

ONE of the most striking features of modern research on growth is the increasing emphasis which is being placed upon the importance of fats in the dietary, not merely from the standpoint of their calorific value, but also as constituting or being associated with substances essential to growth.[1] The view has recently been advanced that many of these essential substances are to be regarded, not as nutritive materials properly so called, but as catalysors of the growth-process,[2] since substances of a fatty nature or commonly associated with fats exist in quantities too minute to be of any nutritive significance yet they are found decidedly to influence the rate of growth in various tissues.

Three of the lipoids especially concerned in the process of growth are cholesterol, lecithin, and tethelin. Although cholesterol is usually classed with the lipoids on account of its solubilities and its invariable association with fatty substances, it is probably a secondary alcohol. Lecithin and cholesterol have been extensively studied. Both are found in nearly all the tissues of the animal body, neither are found in their identical form in plants, although the isomer of cholesterol and substances closely related to lecithin are widely distributed in plants.

Many theories have been advanced as to the function of lecithin. It was suggested by Loew (1899) and again by Reicher (1911) that by the transformation of fatty substances into lecithin, the higher acids are offered to the cells in a soluble form, the same molecules of glycerophosphoric acid serving repeatedly as vehicles for oxidation of molecules of fatty acids; or, in other words, the physiologic oxidation of fats in general is made possible only through combination in lecithin. A number of investigators have suggested that lecithin is accumulated in anticipation of a rapid cell multiplication as the lecithins are most abundant where growth is the most active, and they are decreased as the seat of growth shifts to some other tissue. This is illustrated by the comparative richness in lecithin of the marrow of young bones and its abundance in gland cells, eggs, and spermatozoa. Much experimenting has also been done on the effects of lecithin and other phosphorus compounds on the function of intracellular ferments and on digestion, but in these phases there has been no definite conclusion. However, it has been demonstrated that lecithin, administered by mouth or subcutaneously, is taken up at once by the tissues for their growth.

Yolk of egg is rich in lecithin, and young animals fed on egg yolk grew more rapidly and with better bone development than the control animals (as demonstrated by Cronheim and Müller).

Cholesterol is found in all animal fats and in nearly all the body tissues and fluids. Like lecithin, it is relatively more abundant in the brain than in the other

*From the Departments of Surgery and Biochemistry of the University of California Medical School, San Francisco, Cal.

organs. Cholesterol forms from twenty to as much as ninety per cent of gallstones.

The numerous recent estimations of the cholesterol content of the blood in pregnancy in its relation to cholelithiasis; in diabetes showing a constant high content, and in pernicious anemia showing a constant low content, leads us to expect that the study of the lipoids will modify the present prognosis of these diseases.

That cholesterol is of much importance in the body's economy is shown by the fact that cholesterol is a constant constituent of all the cells and, when these cells are broken down in life processes, the cholesterol is not excreted but is utilized again in the formation of new cells. According to Kusumoto, one function of the liver is to break down dead cells, the cholesterol of which passes with the bile into the intestine. The cholesterol is reabsorbed and carried with the chyle via the thoracic duct to the blood and so to the various tissues for recombination into the constitution of new cells; thus cholesterol is seemingly accumulated by lack of elimination. With age, the cholesterol content of the body increases. This fact is strongly suggestive when considered with the increased tendency to carcinoma as age advances.

Experiments with the Flexner-Jobling carcinoma in rats have shown that cholesterol definitely accelerates the growth of this carcinoma.[3] But the multiplex character of the growth of animals is shown by the fact that cholesterol retards the normal growth of young mice although it accelerates the growth of the postadolescent mice. Lecithin in opposition to cholesterol, retards the growth of the Flexner-Jobling carcinoma in rats, but accelerates the normal growth of tadpoles, both in weight and linear dimensions.[4]

For the past thirty years, growth has been considered more or less under the influence of the pituitary body. Marie, in 1886, associated gigantism with an oversecretion of the pituitary body during youth, and acromegaly with an overactive secretion during maturity. Since the time of Marie's clinical observations and the first extirpation of the pituitary in the same year by Victor Horsley, there has been a great amount of experimentation in which the work of Cushing is preeminent; and while some very valuable facts were disclosed concerning the posterior lobe of the pituitary, the function of the anterior lobe remained hazy.

The pituitary body develops at the extremity of the notochord and contains rudiments of its origin from ectoderm, mesoderm, and entoderm. The striking feature in the development of the pituitary body is that it originates at the meeting point of that portion of the ovum from which the nervous centers, the alimentary canal, the mouth, and the base of the skull are developed. The posterior lobe of the pituitary develops as the infundibulum and is nervous in origin, and, while it has a marked influence on blood pressure and the contractions of smooth muscles, it is not essential to life. The anterior lobe arises from the pharynx or upper end of the primitive intestine, is distinctly glandular in type, resembling the parathyroids in structure, and it is essential to life.

Extracts or emulsions of the anterior lobe cause a retardation of growth in young animals,[5] but in postadolescent animals growth is accelerated. Extracts

or emulsions of the anterior lobe also cause an acceleration in the growth and an increase in the malignancy of the Flexner-Jobling carcinoma in rats.[6] After considering the action of the lipoids—lecithin and cholesterol—it was a logical hypothesis that the chemical substance of the anterior lobe which influences growth is a lipoid. Following up this idea, Robertson, in 1916, published a series of articles in which he described a lipoid which he had isolated from the anterior lobe of the pituitary body. Each pituitary yields about 10 milligrams of this substance. It is soluble in alcohol and ether, and, like lecithin, is a fatty substance in combination with phosphorus and nitrogen. Its phosphorus content is 1.4 per cent and for every atom of phosphorus there are four of nitrogen.[7]

This lipoid influences growth in the same way as extracts or emulsions of the anterior lobe of the pituitary; that is, it apparently retards growth when administered to animals before the age of adolescence, and accelerates growth in the postadolescent period.[8] It increases the rate of growth of the Flexner-Jobling carcinoma in rats in the same way as whole anterior lobe tissue.[9] In view of these experimental results, Robertson suggests that this lipoid is the active principle of the anterior lobe in its influence on growth. It was termed tethelin from τεθηλώς "growing." The effect of tethelin on the repair tissue was studied by experiments on mice wounded by the excision of small pieces of skin.[10] Tethelin was administered hypodermically and it showed a stimulating action on tissue repair.

No work has yet been published on the effect of tethelin as an accelerator of repair in cases of delayed union of fractures, but reasoning from the basis that acromegaly is caused by a hypersecretion of the anterior lobe of the pituitary, it would appear that tethelin would have a very great value in certain cases of delayed union. And may we not anticipate less tedious results in the various forms of skin grafting, also in treating gastric ulcers, and in fact in almost all epithelial lesions tending to chronicity.

Following the suggestion received by the experimental work on wounds in mice, it was decided to use tethelin in the treatment of several types of indolent ulcers, the results of which are given below. With one exception, it was not used in any case where the usual ointments and stimulators of granulation tissue had not been extensively tried without success; nor was it used in ulcers for which there is a well-recognized and efficacious treatment—as in luetic ulcers.

The experiments of C.L.A. Schmidt have shown that tethelin is nontoxic and nonantigenic, hence it is therapeutically without danger to the patient.[11]

To reach the limits of the pharmacopeia and to exhaust the patience of the physician probably nothing can surpass certain types of ulcers, and especially when the cases are ambulatory. With leg ulcers, rest is usually of the utmost importance but many patients are unwilling and many more are unable to secure continued rest in bed for a sufficient time.

As each person has a different physiology, so each ulcer differs from every other; some ulcers refuse to heal under treatment that has sufficed in other cases which appear to be similar. All that is needed in some cases is a soothing dressing and a choice may be made from a long list of ointments—zinc oxide,

boric, Lassar's paste, bismuth paste, vaseline, etc. For stimulation there is also a goodly number to choose from—silver nitrate, iodine, napthalene flakes, balsam of Peru scarlet red. The list of wet dressings is equally long. There is likewise great variation in dressings in which the antiseptic feature is prominent—from a solution of iodine and alcohol to Dakin's solution.

The following cases were selected because to each had been applied a variety of common remedies without success, which fact served somewhat as a control.

All the cases were treated in the out-patient department of the University of California Medical School.

Miss S. O., stenographer, age twenty-seven years. Entered surgical clinic, August 23, 1917.

Diagnosis.—Chronic pyogenic granuloma on finger.

History negative except that two years before she had vomited a small quantity of blood after several hours of dancing. No medical diagnosis available of her condition at that time. She recovered in a few days and there has been no recurrence.

On the first of July, 1917, a wart on her finger became infected by a splinter. After the infection subsided, an ulcer resulted which did not heal although under the treatment of a physician. After a duration of seven weeks, patient entered the surgical clinic.

Examination showed an ulcer 1½ cm. in length on the extensor surface, middle phalanx of middle finger of left hand. The ulcer had a fibrous margin and base of gray, soft granulation tissue. Under nitrous-oxide anesthesia, ulcer was curetted August 23; dry gauze dressing applied, followed in twenty-four hours with zinc oxide ointment dressing daily. August 29 the ulcer was irregular in shape, 0.9 cm. wide in lower portion and 0.6 in upper portion, and 1.6 cm. in length. Granulation beginning to appear but surface of ulcer still concave. Gauze wet with a solution of 100 milligrams of tethelin in 5 c.c. of sterile distilled water.

Second Treatment.—August 31, powdered tethelin, 100 mg. used.

Third Treatment.—September 1, powdered tethelin 100 mg. Granulations level with skin and thin line of epithelium growing in. Ulcer 1.5 and 0.5 in width and 0.8 in length.

Fourth Treatment.—September 3, powdered tethelin, 100 mg. Upper portion of ulcer which was 0.5 in width healed, and lower portion now 0.5 cm. in diameter.

Fifth Treatment.—September 5. Ulcer now 0.3 in diameter and healing.

Patient not seen again until September 11 because of disturbances in the city's street car service. But when seen, September 11, the ulcer was covered with a scab formed of powdered tethelin and epithelium. This was removed. The ulcer was entirely healed. Patient was seen one and one-half months later; the ulcer had remained healed and no scar was visible.

E. T., farmer, age twenty-nine years.

Diagnosis.—Varicose ulcer.

Entered surgical clinic, August 15, 1917.

History.—Twelve years ago had had typhoid fever resulting in a phlebitis in legs. Legs had remained somewhat enlarged since that time. In May, 1917, infected by insect bite followed by scratching, which resulted in an ulcer. Ulcer did not heal. Three months later, Aug. 8, 1917, he entered the University of California Hospital and the ulcer was curetted. Wassermann negative. Transferred to the out-patient department, Aug. 15, 1917. Ulcer dressed in surgical clinic with Lassar's paste. Under this treatment ulcer was healing slowly. After two weeks of this treatment, August 29, when tethelin was first used, the surgical condition was as follows: On inner and anterior surface of left leg, halfway between knee and ankle was an ulcer 1 cm. in diameter, base filled with healthy granulation tissue, but still slightly below the level of the skin. Area about ulcer indurated, veins varicosed.

First Treatment.—August 29, ulcer 1 cm. in diameter. 100 mg. of tethelin in 5 c.c. sterile distilled water on gauze applied to ulcer.

Second Treatment.—August 30, ulcer was covered with epithelium except for small center of 2 mm. The epithelium appeared to lie in several layers and was comparable to a growth of rapidly growing bacteria as staphylococcus. Wet dressing of tethelin 100 mg. again used.

Third Treatment.—September 1, ulcer covered with epithelium and surface slightly convex. Powdered tethelin applied.

September 3. Ulcer healed. Patient discharged.

W. A., waiter, age forty-two years.

Diagnosis.—Indolent pyogenic ulcer.

Entered surgical clinic Sept. 17, 1917.

History—Patient entered Skin Clinic August 20, 1917. Complaint—ulcer on leg. Three weeks previously, infected leg and ulcer resulted. Upon entering skin clinic ulcer was 4 cm. in diameter. Diagnosed as pyogenic ulcer and the surrounding area as an impetiginous dermatitis. Wassermann taken in June, 1917, at a private laboratory, was negative. The ulcer was treated in the skin clinic with Lassar's paste and ammoniated mercury. Patient was given a prescription of ammoniated mercury and diachylon ointment to be applied twice a day. Under this treatment the ulcer healed rapidly for the first week but after that it healed more slowly, and finally during the last week remained fairly stationary.

On September 17, after four weeks in the clinic, patient was transferred to surgical clinic for treatment with tethelin. The ulcer was at that time but 1x1½ cm. and seemed to be healing, although it was recorded as being about that size for a week past.

September 17, wet dressing, 100 mg. tethelin applied.

September 18, same treatment. Granulations healthy.

September 19, powdered tethelin. Granulations level with skin and epithelium growing in.

Following the treatment with the powdered tethelin the granulations immediately broke down. The two cases preceding this had been treated with tethelin powder with good results but this particular ulcer was set back by it, probably on account of the irritation due to contamination of the preparation by a small proportion of inorganic salts (sodium sulphate). Tethelin in solution was again used and in 24 hours the granulations were again healthy. The solution was continued for the next week and although the granulations continued of healthy appearance, the epithelium failed to grow.

October 5, 100 mg. tethelin was injected beneath ulcer and a dry gauze dressing used.

October 8, second subcutaneous injection given beneath ulcer which in the past three days had decreased one-half in size.

October 12, third subcutaneous injection given beneath ulcer. Blood for Wassermann taken to complete record.

October 15, ulcer healed. Wassermann one-plus. During the time the patient was in attendance at the clinic, he was working as a waiter, which employment is least suited to the healing of an ulcer.

C. G., schoolgirl, age sixteen years. Entered surgical clinic Oct. 20, 1917.

Diagnosis.—Ulcer on leg.

Two months before entering the clinic, she struck her shin on a box. Hematoma formed, became infected and ulcer resulted. Two months of treatment by a physician before entering clinic.

Ulcer at first examination in clinic was irregular in shape, 2 cm. in diameter, deeply sunken below level of skin; unhealthy base covered with sluggish granulations, thickened margin. Ulcer situated on crest of tibia about 12 cm. above ankle.

Subcutaneous injections of tethelin, 100 mg. were given along margin of ulcer until red granulations appeared. Then tethelin used as a wet dressing. Ulcer continued to heal.

October 31, tethelin mixed with lanolin applied to ulcer. Several days later, patient fell on school ground, bruising ulcer. After November 15 tethelin no longer used, ulcer was healed all but 5 mm. Patient given lanolin to use at home.

J. N., Italian girl, age sixteen years. Entered surgical clinic May 29, 1916.

Diagnosis.—Ulcer on leg.

History.—Ulcer began eleven years ago, 1906, when patient was six years old. It began near the right internal malleolus but spread upward, gradually involving nearly all of the anterior aspect of the leg. Not treated for one year; then operated on by a surgeon in Tennessee, who removed part of the middle third of the tibia. Wound not entirely healed until two years later. Remained healed for nearly seven years, but in November, 1915, after trauma to leg, a small ulcer appeared on the site of the old operative scar. This second ulcer gradually increased in size.

When patient entered clinic, six months after ulcer appeared, there was a large irregular ulcer on inner surface of upper and middle third of right leg, with a punched-

out border and slight purulent discharge. Wassermann in blood at this time negative. Ulcer was dressed with ammoniated mercury ointment and patient given potassium iodide and mercuric chloride.

During the next three months, until September 2, 1916, the patient continued mixed treatment and the ulcer received a variety of medications, including balsam of Peru and zinc oxide ointment, aristol, strapping with zinc oxide tape, and ammoniated mercury ointment; the last being used the greater part of the time but the ulcer showed no constant tendency to heal. After this period, Sept. 2, 1916, the same dressings were continued but more vigorous luetic treatment was adopted. Though the Wassermann had been negative and radiograms of the bones of the other leg, the clavicles and the humeri were also negative, yet a radiogram of the right leg showed the tibia to be somewhat enlarged and bowed. It was of course impossible to estimate how much of this bowing and enlargement was due to the operative measures at seven years of age.

The more energetic luetic treatment consisted of ½ c.c. of mercuric salicylate, of 20 per cent strength, injected once a week into the gluteal muscles. In the record is noted a remarkable improvement in the ulcer after two administrations of the mercuric salicylate, but a month later it was recorded that, although the wound was covered with healthy granulations, the epithelium grew very slowly. Two months after the mercuric salicylate was begun, potassium iodide by mouth was administered up to 45 gtt. daily. Nearly four months after the first treatment with he mercuric salicylate, the patient was discharged from the surgical clinic, the ulcer being healed, Dec. 26, 1916. The potassium iodide was discontinued but the mercuric salicylate was continued. The ulcer remained healed eight weeks until February, 1917, when the skin was again traumatized and a new ulcer formed on the old scar. The administration of mercuric salicylate was continued but the ulcer continued to increase in size. On March 13, 1917, potassium iodide was resumed and gradually increased to 60 gtt. daily. Salvarsan was also added to the treatment. The ulcer continued to show no constant tendency to heal.

From August 16, 1917, the local treatment of the ulcer was augmented by daily exposures to alpine light for ten to twenty minutes and dressed with Lassar's paste (without salicylic acid). The alpine light was continued for one month, during which time the ulcer showed some improvement.

On Sept. 13, 1917, all luetic treatment and the alpine light were stopped, and treatment with tethelin commenced. During the year, from Sept. 2, 1916, to Sept. 13, 1917, the patient had received 34 injections of ½ c.c. of mercuric salicylate in 20 per cent strength, and, for the greater part of the time, a saturated solution of potassium iodide up to 60 gtt. daily, also four intravenous injections of salvarsan. And during that year of luetic treatment, the ulcer had been well for a period of eight weeks only.

At the time, Sept. 13, 1917, when all luetic treatment was stopped and treatment with tethelin begun, there were two ulcers close to the inner margin of the crest of the tibia in the middle third of the leg. The upper ulcer was of crescent shape, 3.5 cm. in length and its greatest width was 1 cm. The granulations were uneven and the margin ragged and unhealthy. The lower ulcer, lying 1 cm. inferiorly was 2.1 cm. in length and 1.8 cm. in width, was healthy and the epithelium was growing in. The ulcers were extremely sensitive to touch.

For one week, gauze wet with a solution of 200 mg. of tethelin in 10 c.c. of sterile distilled water was daily applied to the wound. For the first four days, there was a marked improvement but by the end of the eighth day the new epithelium was breaking down in one area as fast as it grew in another. Powdered tethelin was then used and this proved too irritating, and the granulations broke down. For a week no tethelin was applied but aristol was dusted over the ulcer. Under this treatment the ulcer increased in size and lost all appearance of healing. At the end of a week of treatment with aristol, Oct. 3, 1917, the two ulcers had united forming an area 7 cm. in length and 2 cm. in width; the granulations were uneven, there was considerable secretion, and the patches of epithelium were defoliating. On Oct. 3, subcutaneous injections of 100 mg. of tethelin were given near the margin of the ulcer. Dry gauze dressing. This treatment continued for one week; ulcer improved but the dry gauze injured the new epithelium. On Oct. 10, therefore, wet dressings of 100 mg. in 10 c.c. of sterile distilled water (twice the dilution before used) were employed in addition to the subcutaneous injections of tethelin. Two weeks later, although the ulcer was healthy and healing, it was thought to lessen trauma to the new tissue by using a mixture of tethelin in some fatty substance. Lanolin was chosen, as the tethelin is remarkably hygroscopic and lanolin capable of dissolving water. This mixture worked remarkably well. On Nov. 17, 1917, the ulcer was but 3½

cm. in length. On Nov. 22, 1917, it was but 1 cm. in diameter, there being a reduction in length from 7 cm. to 1 cm. in six weeks.

On Nov. 30, the patient called at the clinic, after a holiday, with the skin lacerated just below the knee and the area of ulceration bruised. No information could be obtained as to how the leg was injured. However, the injury was of no permanent consequence.

During December, lanolin only was used as the available supply of tethelin had been exhausted. On Dec. 30, the ulcer was 0.7 cm. in length and 0.3 cm. in width, having remained nearly stationary for a period of four weeks. Jan. 2, 1918, a few tubes of tethelin were obtained, and the ulcer given two successive treatments with tethelin. By Jan. 7, 1918, the ulcer had healed and the case was dismissed. Four weeks later, Feb. 2, the patient reported to the clinic. The ulcer had remained healed.

There is no doubt that this ulcer will recur if the crest of the tibia is traumatized. When healed, the epithelium over an area of 27 cm. is closely adherent to the periosteum of the tibia; the blood supply is very deficient, there is no subcutaneous tissue, and unless this portion of the leg is covered by a protective dressing, the ulcer will sooner or later recur.

There has been a certain satisfaction in healing this ulcer with tethelin as it was nearly a year's duration and had run the whole gamut of ulcer treatment.

In a case of Basin's disease in a child six years old, tethelin was also tried. Here there were several ulcers and a control could be had. Tethelin was tried for two weeks without success. Other forms of local treatment were also unavailing until the patient was given bichloride baths.

Mrs. H., age sixty years. Admitted to clinic Oct. 28, 1917.

Complaint—Several ulcers in old scar.

History dating to childhood. No Wassermann taken. Solution of tethelin used over a period of two weeks. The inflammation surrounding the ulcers subsided and one small ulcer showed new granulation tissue but after two weeks of treatment the patient did not return to the clinic.

D. E., age eleven years. Treatment begun Aug. 29, 1917.

Tethelin was used in solution on a healthy operative wound on the thigh over an area from which a Wolfe graft had been taken. In two treatments the granulations became exuberant and had to be cauterized with silver nitrate. As there was no control, the results in this case seem only to have shown that tethelin did not cause a retardation of the growth of the granulation tissue.

SUMMARY

1. The significance of lipoids in the various processes related to growth is discussed.

2. Evidence is advanced of the efficiency of tethelin, a lipoid extracted from the anterior lobe of the pituitary body, in the treatment of chronic ulcers.

BIBLIOGRAPHY

[1]Hopkins, F. G.: Jour. Physiol., 1912, xliv, 425.
McCollum, E. V., and Davis, M.: Jour. Biol. Chem., 1913, xv, 167.
MacArthur, C. G., and Luckett, C. L.: Jour. Biol. Chem., 1915, xx, 161.
[2]Robertson, T. B.: Jour. Biol. Chem., 1916, xxiv, 347, 363, 385, 397, 409; Ibid., 1916, xxv, 635, 647, 663; Endocrinology, 1917, i, 24.
[3]Robertson, T. B., and Burnett, Theo. C.: Jour. Exper. Med., 1913, xvii, 344.
[4]King, H. D.: Biol. Bull., 1907, xiii, 40.
Johnson, M. S.: Univ. of California Publ. Zoology, 1913, xi, 53.
[5]Aldrich, T. B.: Amer. Jour. Physiol., 1912, xxxi, 94.
Schafer, E. A.: Quart. Jour. Exper. Physiol., 1912, v, 203.
Wulzen, R.: Amer. Jour. Physiol., 1914, xxxiv, 127.
Robertson, T. B.: Jour. Biol. Chem., 1916, xxiv, 385.
[6]Robertson, T. B., and Burnett, Theo. C.: Jour. Exper. Med., 1915, xxi, 280.
[7]Robertson, T. B.: Jour. Biol. Chem., 1916, xxiv, 409.
[8]Robertson, T. B.: Jour. Biol. Chem., 1916, xxiv, 397.
[9]Robertson, T. B., and Burnett, Theo. C.: Jour. Exper. Med., 1916, xxiii, 631.
[10]Robertson, T. B.: Jour. Amer. Med. Assn., 1916, lxvi, 1009.
[11]Schmidt, C. L. A.: Jour. Lab. and Clin. Med., 1917, ii, 711.

THE GONOCOCCIDAL ACTION OF PROTEIN SILVER SOLUTION IN VITRO*

With Special Reference to the Silver-Fastness of Gonococci

By Harry Culver, M.D., Chicago, Ill.

THE ideal therapeutic agent for a local infection is one which has an elective action against the microorganisms without doing damage to the tissues. Numerous chemical agents when in sufficient concentration *in vitro*, have marked destructive action on many pathogenic bacteria but the concentration contraindicates their use in infected tissues.

The gonococcus has very little resistance to many ordinary chemicals used as germicides, its tolerance to protein silver salts being so meager that these substances might be known as specifics for gonococcal infection, were it possible for the silver solution in proper concentration to come in contact with all gonococci in the tissues. However, it has been repeatedly demonstrated, both experimentally and clinically, that after thirty-six to forty-eight hours after the inoculation of the mucous membrane with gonococci the organisms have penetrated between the epithelial cells and lie safely protected in the submucosal connective tissues. Chemicals which would reach, and destroy gonococci in the urethra, or even those between the epithelial cells, may not have the penetrating power necessary to reach the submucosa, or may reach it so diluted by tissue juices, that the concentration is not sufficient to kill the organisms with which they come in contact.

Scholtz* has found that by injecting the urethra of a man suffering from acute gonorrhea, with protargol 1%-3% argentamine or silver nitrate 1 to 3000, and holding it for 20 to 30 minutes, after which the medicament was removed by urethral irrigation, the surface epithelium, removed with a curette, contained many intra- and extracellular gonococci which could not be artificially cultivated. He therefore concluded that the organisms of the surface mucous membrane were dead. He also found by similar methods that the deeper mucosa and submucosal tissues contained living, cultivatable organisms.

The number of silver preparations now being used is large. From their chemical structure and reported therapeutic worth, it must be concluded that they all have more or less value as gonococcidal agents. One is frequently confronted with the question of relative gonococcidal strength of these various silver preparations. Certain strengths of each of these salts are recommended by manufacturers for clinical use, but no publications are available of the relative activity of these preparations *in vitro*, therefore four commonly used silver salts argyrol, silvol, protargol, and nargol were taken as being a fairly representative list from this group for standardization.

*From the Department of Pathology and Bacteriology, University of Illinois, College of Medicine, Chicago, Ill.

*Prinzipien der Gon-Behandlung, Deutsch. med. Wchnschr., 1905, No. 23, p. 935.

TECHNIC

Ten small test tubes are placed in a rack and in each is put a standard amount of sterile hydrocele broth. A determined amount of freshly prepared silver solution is added to each tube to make the silver in the following dilutions 2%-1%-½%-¼%, etc., to 1/128% with one tube of hydrocele broth as a control. To each of these tubes is added a standard amount of a suspension of a 24-hour growth of gonococci. As the organisms are added the tube is agitated to get an even mixture and at once a drop of this mixture is inoculated on a long blood agar slant. The slant is covered with either the water of condensation, already in the tube, or a few drops of sterile water to dilute the gonococcidal agent brought over with the organisms. Similar tube inoculations are made after the various periods of exposure of the gonococci to the gonococcidal agent, which periods were 5 min., 10 min., 30 min., and one hour. All inoculated tubes were incubated at 37° C. for 48 hours and the colonies on the tubes were counted or approximated. To be relatively certain of the accuracy of the results, these experiments were repeated six times for each silver salt represented, and Table I represents average experiments selected from the above mentioned list.

TABLE I

SHOWING THE RELATIVE ACTIVITY OF ARGYROL, PROTARGOL, SILVOL AND NARGOL AGAINST GONOCOCCI IN VITRO

ARGYROL	AT ONCE	5 MIN.	10 MIN.	30 MIN.	1 HR.
2%	0	0	0	0	0
1%	0	0	0	0	0
½%	4	0	0	0	0
¼%	11	10	0	0	0
⅛%	10	12	25	0	0
1/16%	25	10	20	25	0
1/32%	200	400	10	25	10
1/64%	200	50	15	500	200
1/128%	1000	100	400	100	200
Control	2000	1000	1000	1000	1500

SILVOL	AT ONCE	5 MIN.	10 MIN.	30 MIN.	1 HR.
2%	0	0	0	0	0
1%	0	0	0	0	0
½%	0	0	0	0	0
¼%	100	100	0	0	0
⅛%	50	10	15	0	0
1/16%	100	10	25	0	0
1/32%	500	200	250	0	0
1/64%	200	2000	2000	100	0
1/128%	3000	2000	3000	3000	0

PROTARGOL	AT ONCE	5 MIN.	10 MIN.	30 MIN.	1 HR.
2%	0	0	0	0	0
1%	0	0	0	0	0
½%	0	0	0	0	0
¼%	0	0	0	0	0
⅛%	50	25	0	0	0
1/16%	50	10	15	0	0
1/32%	500	50	200	0	0
1/64%	300	250	100	100	0
1/128%	1000	200	1000	50	100

NARGOL	AT ONCE	5 MIN.	10 MIN.	30 MIN.	1 HR.
2%	0	0	0	0	0
1%	0	0	0	0	0
½%	0	0	0	0	0
¼%	0	0	0	0	0
⅛%	50	100	0	0	0
1/16%	1000	40	0	0	0
1/32%	1000	500	11	0	0
1/64%	2000	50	400	11	0
1/128%	2000	1000	1000	500	100

From this table it can readily be seen that beyond a certain concentration in all of these silver salts an instantaneous exposure is sufficient to kill all gonococci with which they come in contact. This concentration is also seen to be well below that usually used of the same silver solution clinically. How-

ever the exposure of unprotected organisms to the action of germicides *in vitro* is very different from the application of a similar strength of germicide *in vivo* where the organisms may be inaccessible and protected by diluting tissue juices. Nevertheless, the relative action of these silver solutions *in vitro* represents their probable relative action *in vivo*, since their penetrating qualities are not known to differ.

From this table, one must conclude that protargol and nargol have practically the same gonococcidal power, since they both kill in ¼ per cent concentration both on exposure for an instant and also for a five-minute exposure. There is further equality of action demonstrated by longer exposures. Likewise argyrol and silvol are seen to be almost equal in gonococcidal power, the slight advantage being in favor of silvol, since it kills at ½ per cent both on instantaneous exposure and five minutes' exposure; while argyrol does not completely sterilize instantaneously in ½ per cent concentration, but does so in five minutes. From a careful analysis of this table then, it is seen that protargol and nargol, equal in gonococcidal activity, are about twice as active as argyrol and silvol.

EFFECT OF EXPOSURE TO LIGHT AND AGE OF SOLUTION

The above silver salts were prepared in 2 per cent solutions exposed at room temperature to ordinary daylight for two months. The gonococcidal activity of these solutions was tested at various intervals during the two months.

Table II represents the action of these various silver salts at the end of two months.

TABLE II

SHOWING THE ACTION OF OLD SILVER SOLUTIONS ON GONOCOCCI
(Compare with Table I.)

ARGYROL	AT ONCE	5 MIN.	10 MIN.	30 MIN.	1 HR.	SILVOL	AT ONCE	5 MIN.	10 MIN.	30 MIN.	1 HR.
2%	15	0	0	0	0	2%	20	0	0	0	0
1%	50	40	0	0	0	1%	25	0	0	0	0
½%	50	20	10	0	0	½%	25	20	0	0	0
¼%	25	20	50	0	0	¼%	25	200	50	10	0
⅛%	200	25	20	10	10	⅛%	200	50	10	10	0
1/16%	500	200	200	200	50	1/16%	100	100	50	50	50
1/32%	200	500	200	500	25	1/32%	100	200	50	20	10
1/64%	500	1000	500	500	25	1/64%	500	100	100	50	200
1/128%	1000	1000	1000	1000	1000	1/128%	500	500	500	500	500
Control	2000	2000	2000	2000	1000						

PROTARGOL	AT ONCE	5 MIN.	10 MIN.	30 MIN.	1 HR.	NARGOL	AT ONCE	5 MIN.	10 MIN.	30 MIN.	1 HR.
2%	10	0	0	0	0	2%	20	0	0	0	0
1%	15	0	0	0	0	1%	20	0	0	0	0
½%	40	0	0	0	0	½%	20	0	0	0	0
¼%	50	10	0	0	0	¼%	500	200	0	0	0
⅛%	1000	100	10	0	0	⅛%	2000	100	0	0	0
1/16%	1000	100	10	0	0	1/16%	1000	150	100	0	0
1/32%	1000	200	100	10	0	1/32%	1000	500	200	100	0
1/64%	2000	1000	500	100	10	1/64%	2000	2000	25	100	0
1/128%	2000	2000	2000	2000	1000	1/128%	2000	2000	2000	2000	1000

From a comparison of Tables I and II it is seen that there is a definite weakening of all the silver solutions tested. This is especially marked in the case of argyrol since its efficiency in a five-minute exposure has decreased 75 per cent, while silvol, protargol, and nargol under the same conditions have each lost one-half of their effectiveness. The loss of strength begins as early as the third day after the solution is made, and from interval experiments similar to that represented in Table II it is found that this decrease in gonococcidal activity is almost directly proportional to the age of the solution. From clinical experience it is well recognized that old solutions are more irritating to an inflamed mucosa than freshly prepared ones. Hence the practice of having stock solutions of silver salts is disadvantageous from more than one standpoint. Also the amounts prescribed for home use by the patient should not exceed a quantity enough for a few days use; or better still where possible, the solution might be prepared daily.

EFFECT OF HEAT

Warm solutions applied to an inflamed mucous membrane are soothing and they also increase the already present hyperemia. These facts together with the fact that gonococci are heat sensitive, make the use of warm silver solutions a logical one. This is in accord with clinical results. The following experiments were made to determine what effect, if any, heat would have on the bactericidal power of the four silver solutions. One per cent solutions were made of nargol, protargol, argyrol, and silvol and at once exposed to a temperature of 120° F. After cooling, the dilutions were made in the usual way and their action on gonococci determined as seen in Table III.

TABLE III

EFFECT OF SILVER SOLUTIONS (AFTER HEATING TO 120° F.) ON GONOCOCCI IN VITRO

ARGYROL	AT ONCE	5 MIN.	10 MIN.	30 MIN.	SILVOL	AT ONCE	5 MIN.	10 MIN.	30 MIN.
2%	0	0	0	0	2%	0	0	0	0
1%	2	0	0	0	1%	10	0	0	0
½%	25	0	0	0	½%	10	0	0	0
¼%	25	10	0	0	¼%	50	25	0	0
⅛%	40	25	2	0	⅛%	100	5	0	0
1/16%	50	500	25	0	1/16%	100	100	25	10
1/32%	500	300	2000	500	1/32%	25	2000	2000	0 (?)
1/64%	2000	5000	200	2000	1/64%	1000	2000	2000	500
1/128%	3000	5000	5000	5000	1/128%	2000	2000	2000	2000
PROTARGOL	AT ONCE	5 MIN.	10 MIN.	30 MIN.	NARGOL	AT ONCE	5 MIN.	10 MIN.	30 MIN.
2%	0	0	0	0	2%	0	0	0	0
1%	0	0	0	0	1%	0	0	0	0
½%	1000	0	0	0	½%	1000	0	0	0
¼%	500	500	0	0	¼%	200	0	0	0
⅛%	2000	100	?	0	⅛%	200	100	0	0
1/16%	300	50	25	4	1/16%	1000	50	25	2
1/32%	2000	1000	10	25	1/32%	0 ?	0 ?	25	10
1/64%	2000	2000	2000	25	1/64%	1000	500	500	?
1/128%	2000	2000	2000	1000	1/128%	1000	2000	1000	2000

A comparison of Tables I and III reveals certain apparent facts. Heat tends to reduce the gonococcidal strength of all of the silver solutions tested. This reduction is demonstrated, however, only in the first column where the exposure was for an instant. For argyrol, silvol, and nargol there is no perceptable decrease in the activity in all other interval exposures, while protargol shows a distinct reduction in killing power for every time interval used in the experiment. However, the gonococcidal power of heat, the increased hyperemia produced by, as well as the increased penetrating power of, hot solutions more than compensate for the slight decrease in germicidal strength. Nevertheless repeated heating of the same solution would undoubtedly render it worthless as a gonococcide; hence it would be well not to keep these solutions once they have been heated. Especially is this true of protargol.

CHEMICAL-FASTNESS OF GONOCOCCI

Gonococcal infection is encountered which does not respond to repeated application of the same silver solution or any single germicide used locally for that purpose, while immediate response may be observed if the germicide is changed to another member of the same group or one from a different group. This occurrence would lead one to suspect that the gonococci in the deeper tissues have acquired a resistance for the first medicament, by dint of repeated application to these gonococci in the same or increasing concentration, but not of sufficient concentration to kill, this acquired tolerance being applicable only to the particular medicament used and not to other members of the same group, or different groups. Seinsi and Noguchi* have found that the tolerance of the Treponema pallida can be raised to salvarsan to five and one-half times their original tolerance by growing these organisms in gradually increasing strengths of arsenic preparations. In a similar manner they found their tolerance for mercury was raised thirty-five to seventy times. Danyz,† working with anthrax bacilli and arsenic preparations likewise found an acquired tolerance for these organisms against such preparations. In similar experiments gonococci were grown in hydrocele-broth tubes to which had been added various percentages of silver solutions used in the above experiments. These organisms were left in contact with the silver solution media from 30 minutes to one hour, and then plated on blood-agar tubes. The growth from the blood-agar tube, which corresponded to the highest silver solution was used in a similar experiment. By thus transferring gonococci from a silver solution media to a similar media of greater silver concentration, organisms were grown which had a marked increased resistance for this particular silver solution. This silver tolerance is gradually acquired. In the experiments here reported the adaptation took place in the course of two weeks and usually from five to six successive transfers to produce the results shown in Tables IV and V.

*The Drug Fastness of Spirochetes to Arsenic, Mercurial and Iodide Compounds in Vitro, Jour. Exper. Med., 1917, xxv, 349.

†Immunisation de la Bacteridie charbenneuse contre L'Action du Serum du Rat, Ann. de l'Inst. Pasteur, 1900, xiv, 641.

TABLE IV

REPRESENTS THE ACTION OF ARGYROL, SILVOL AND NARGOL ON STRAINS OF GONOCOCCI IMMUNIZED AGAINST THESE SILVER SOLUTIONS

ARGYROL	AT ONCE	5 MIN.	30 MIN.	SILVOL	AT ONCE	5 MIN.	30 MIN.	1 HR.	NARGOL	AT ONCE	5 MIN.	30 MIN.
1/10%	4000	2000	1000	1/20%	200	200	10	0	1/20%	3000	1000	1000
1/5%	2000	2000	0	1/10%	100	25	20	0	1/10%	3000	2000	2000
1/2%	2000	500	0	1/2%	100	200	10	0	1/5%	50	20	400

TABLE V

REPRESENTS THE ACTION OF NARGOL AND PROTARGOL AGAINST A NARGOL-TOLERANT GONOCOCCUS

NARGOL	AT ONCE	5 MIN.	30 MIN.	PROTARGOL	AT ONCE	5 MIN.	30 MIN.
1/20%	3000	1000	1000	1/20%	1000	100	10
1/10%	3000	2000	2000	1/10%	50	10	0
1/5%	50	20	400	1/5%	0	0	0

The increased resistance acquired by the gonococci is not great in any instance; but no doubt this resistance could be further increased by continuing the experiments. However, Table IV compared with Table I gives sufficient evidence that such an adaptation does take place since the silver solutions are only one-half as effective against the treated as against the untreated stain.

The possibility of a general tolerance to all silver solutions must be considered once an organism gains resistance to any one silver solution. In order to investigate this aspect of the question a nargol-tolerant organism (10 per cent in 30 minutes exposure) was tested against various concentrations of nargol and protargol as represented in Table V.

While this particular culture of gonococci exhibits a considerable resistance against the action of nargol; there is no such resistance produced against the action of protargol which acts on this strain of gonococci as it does on an ordinary strain. Similar experiments for organisms treated with other silvers have been made and tested as in Table V, against the same and different silver solutions, with no variance from the general conclusions that may be drawn from this table.

SUMMARY

From repeated experiments *in vitro*, it is found that protargol and nargol are practically equal in effectiveness as gonococcidal agents, while argyrol and silvol are likewise of almost the same strength. The first two being about two times as strong as the last two salts.

A considerable deterioration takes place in each of these silver solutions after exposure to light and age. This is especially marked with argyrol which loses 75 per cent of its gonococcidal strength with such an exposure for two months; while nargol, protargol, and silvol loses 50 per cent of strength under similar conditions.

Heating the solutions of the above-mentioned silver salts reduces their action as chemical gonococcides, which reduction is most marked in protargol, the therpeutic usefulness of physical heat in gonococcus infection, however,

probably more than recompenses the slight gonococcidal reduction of the solutions.

Continuous or intermittent exposure of a culture of gonococci to gradually increasing concentration of any single silver solution *in vitro* produces very apparent increased tolerance in the organisms for the specific silver solution used. This increased tolerance is not general for all protein silver solutions; as any other member of this silver group will act against this drug-fast organism as against any untreated organism. These experimental facts are of considerable clinical value since it is at once apparent that old silver solutions should not be used, also that heating to 120° F. does not reduce the chemical effectiveness of these solutions, however a silver solution once heated could not be repeatedly heated without reducing its therapeutic worth. Gonococcal infection which is very resistant to one particular gonococcide, may be so, due to an increasing tolerance of the infecting gonococci for the particular gonococcide used. The proper clinical result may be obtained by changing the gonococcide to one of the same group or different group.

THE DEMAND FOR AND TRAINING OF LABORATORY TECHNICIANS*

By JOHN A. KOLMER, M.D., PHILADELPHIA, PA.

FOR many years persons who were not graduates in medicine, and especially young women, have been trained in certain laboratory work as in histological methods, particularly in laboratories connected with medical schools and large hospitals and for various other kinds of work in municipal and commercial laboratories. As a general rule the young men and women who have chosen this occupation have proved quite successful and particularly the latter, although opportunities have been quite limited and remuneration comparatively low.

The demand for properly trained laboratory aids or technicians has been steadily increasing in the last few years due, not only to the organization of more municipal laboratories, but more particularly to the establishment of laboratories in hospitals and other institutions for the care of the sick and injured and of private laboratories for individual physicians and surgeons. In Pennsylvania the demand has been particularly heavy because about two years ago the legislature enacted a law requiring all hospitals and institutions, particularly those receiving state aid, to install and equip an adequate laboratory and to employ a laboratory technician on a full-time basis. An inspection of all the institutions of the state by the Board of Medical Licensure, under the presidency of Dr. J. M. Baldy, has shown that the hospitals located in the cities, towns and rural districts, with the exception of relatively few of the larger institutions located in the principal cities, were without adequate laboratory facilities for the study and diagnosis of persons seeking advice and treatment, or for an adequate laboratory service and

*From the Department of Pathology and Bacteriology in the Philadelphia Polyclinic.

training for their internes. All institutions caring for the sick and injured, and particularly those offering interneships, were classified and required by the Board to install a laboratory adequately equipped and conducted by a pathologist and technician; furthermore, the staffs of the respective institutions were urged and requested to avail themselves of these laboratory facilities.

The law of Pennsylvania requires that the recently graduated physician seeking a license to practice medicine and surgery must have graduated from an acceptable school and have served at least one year's interneship in a hospital and that this interneship must have been spent in a hospital acceptable to the Board; one of the requirements exacted by the Board is that the hospital have a properly conducted laboratory offering a satisfactory laboratory service to the interne. Hospitals failing to install and conduct a laboratory meeting the requirements of the Board of Medical Licensure are refused recognition and their internes are regarded as ineligible for examination for a license to practice medicine and surgery.

This law of Pennsylvania, which marks an epoch in medical education, naturally worked a hardship on many hospitals and particularly the smaller and poorer institutions, but an analysis of the situation shows very clearly the wisdom in demanding a higher standard of education and experience on the part of the physician seeking to practice medicine and surgery among its citizens; in the present day of refined and practical laboratory methods for the diagnosis of disease not a small part of the education of the medical man is embraced in a working knowledge of laboratory methods in the diagnosis and treatment of disease. In our opinion great credit is due Dr. J. M. Baldy, and other members of the Board of Medical Licensure, for the very important work they have done in raising the standards of hospitals and requirements for license in the State of Pennsylvania, the influence of which will be exerted throughout the United States.

Furthermore, since the entry of the United States into the Great War, calling many of our pathologists and bacteriologists into the medical department of the Army and Navy, the demand for laboratory technicians has still further increased; if the war continues this demand for technicians will continue to increase not only for the laboratories of institutions, municipalities and private physicians, but probably also for the laboratories of the various hospitals in the Army and Navy and possibly even for the cantonments in this country and the hospitals abroad.

As a general rule technicians who have been trained in but one phase of laboratory work as in histologic methods, are required only in the larger institutions handling sufficient of this work to occupy the technician's full time; in smaller institutions and for private laboratories a broader training and experience is demanded.

The object of this communication is to emphasize the importance of large laboratories, and particularly those connected with teaching hospitals, to undertake the systematic training of properly prepared young men and women and particularly the latter, in laboratory methods in order to meet this increasing demand for laboratory technicians. During the past two years the Polyclinic

has offered courses of instruction which have proved highly successful; more than seventy-five young women have been prepared and are giving efficient and satisfactory services in the laboratories of hospitals and other institutions throughout the United States.

These courses of instruction are intensive and eminently practical with most attention devoted to technic and practical instruction; lectures are reduced to a minimum in order to devote most of the time to practical work. Instruction is given on five or six days in the week and for four to six hours per day. The classes are limited to a few students in order to permit individual instruction and supervision and criticism of practical work; it has not been found practical or advisable to attempt the teaching of large classes. Each course of instruction is designed to give a broad training in order to enable the technician to handle a varied amount of work as is usually encountered in a well conducted and busy laboratory.

Candidates for instruction must be high school graduates or possess the equivalent in preliminary education; a knowledge of chemistry and biology has been found helpful but not essential. Our most successful students have been those who have entered into this work whole-heartedly and with the purpose of making it their profession; a few have subsequently studied medicine. By reason of their patience, attention to details, cleanly habits and the ability to concentrate most young women in our experience have proved apt pupils and well fitted for work as laboratory technicians. These qualities are frequently lacking in men, whereas they can be developed to a high degree in our students and ensure the conduct of laboratory tests and methods in an orderly, painstaking, accurate and thorough manner. It is the common experience of most pathologists that a well-trained woman technician is capable of doing high-grade work equal to and sometimes superior to that of the physicians themselves and particularly of the interne of average ability and training.

In the Polyclinic laboratories the first course of instruction is in *laboratory technic* which includes systematic training in the preparation of the different kinds of culture media; the different methods of fixing, hardening, embedding, cutting and staining tissues; the preparation of stains; sterilization of glassware and similar work of a fundamental nature. At the completion of the course the student is given a thorough practical and written examination and, if these are passed successfully, the student is eligible for the course in *clinical pathology* which includes instruction in the chemical and microscopical examination of urine, blood, gastric contents, feces and clinical bacteriology, the latter including the Widal reaction, examination of cerebrospinal fluid, sputum, cultures and smears. At the completion of this course a thorough examination is given and it is my custom to advise students to accept positions in order to gain wider experience in the methods and tests which they have been taught, and then to return to the laboratories for instruction in complement-fixation technic with particular reference to the *Wassermann* and *gonococcus complement-fixation tests*. Not infrequently the students elect to continue their instruction until the course in the Wassermann reaction has been covered. I fully realize the great importance of thorough instruction and particularly practical experience of the technician in the conduct of the Wassermann reaction and my experience dur-

ing the past three years, covering the training of sixty-five persons, has shown me that properly qualified young women are readily trained in the preparation and standardization of the various biologic reagents employed in this important and intricate reaction and to conduct the test in a thoroughly reliable manner. Courses of instruction are also offered to persons having laboratory experience, in *bacteriology* and the *preparation of bacterial vaccines* and in *advanced clinical pathology*, the latter course embracing instruction in such subjects as the serologic differentiation of pneumococci; the determination of blood sugar, blood urea and similar chemical methods; the preliminary tests for blood transfusion; the bacteriologic and microscopic examination of milk and water and work of a similar nature. As stated above most attention is devoted to a training in methods and technic, although the students also acquire a fairly good knowledge of the clinical applications and importance of the work in which they are being instructed. When their instruction is finally completed I feel confident of their ability to render valuable and efficient service as technicians, although I constantly advise that it is necessary for them to work under supervision until they have acquired further experience and greater confidence in their work and abilities.

A properly trained and experienced technician is capable of handling a large amount of work of a more or less mechanical nature and thereby conserve the time and attention of the pathologist and physician; up to the present time no attempt has been made to train technicians in pathologic histology because this work demands thorough preliminary instruction in normal histology which is offered only in undergraduate medical schools. Therefore, while technicians are being trained to prepare tissues for microscopical examination and to pass upon the quality of their work by microscopic inspection, they are not being trained to diagnose the tissue changes.

The majority of our technicians have been offered salaries averaging $900.00 a year with maintenance provided in the hospitals in which they are employed; it would appear that this is a fair wage for at least the first year's work. After this time the technician who proves herself worthy, by reason of extended experience and good work, should expect increased remuneration.

It is to be hoped that other states will follow the example of Pennsylvania in requiring her institutions for the care of the sick and injured to offer adequate laboratory facilities for the modern diagnosis and treatment of disease and for the instruction and experience of internes. If each hospital or institution will offer and give the persons seeking their aid, the benefits of laboratory examinations and demand that the physicians and surgeons on the staff shall be acquainted with the possibilities of a good laboratory service in aiding the diagnosis and treatment of disease, the majority of institutions will need a technician; in my opinion it is entirely right and proper to charge persons financially able to afford a private physician, room, and nurse, for the laboratory examinations made in order to meet the expense of maintaining a laboratory and the salaries of pathologist and technician. In order to meet the increasing demands for laboratory technicians it is hoped that laboratories with ample facilities and material, will offer adequate and comprehensive courses of instruction and an abundance of practical work to small classes of properly qualified persons, in order to supply the demand for broadly trained and experienced technicians.

LABORATORY METHODS

HOOVER'S DIAGNOSTIC SIGNS ELICITED FROM THE MOVEMENTS OF THE RIBS*

By R. G. Pearce, B.A., M.D., Cleveland, Ohio

PALPATION and inspection have not played as important a role in the diagnosis of thoracic disease as the methods merit. It is true that most clinicians observe the character of the respiratory movements, any asymmetry of the thorax, the presence or the absence of Litten's sign, or any abnormality of the cardiac impulse. The observations are quickly made, and auscultation and percussion are employed to elicit further pathologic signs. It may be that the invention of methods of percussion and the introduction of the stethoscope have discouraged the use of the eyes and the fingers as instruments of precision. Be this as it may, it is nevertheless true that the older physiologists and physicians are responsible for most of the current teachings of the respiratory movements. We are accordingly ready to welcome any contribution in this field. Recently Dr. C. F. Hoover[1] has reported some very interesting and important physiologic and diagnostic observations on the movements of the upper and lower ribs and the diaphragm in health and in disease.

He calls attention to the fact that the density of the lungs has occupied the attention of the clinician to the exclusion of the no less important attributes of volume and extensibility, and that a definite idea of these latter characteristics affords valuable aid in the diagnosis of pulmonary and thoracic disease, right and left cardiac enlargement, subphrenic and hepatic changes, and paralysis of the intercostal or diaphragmatic muscles.

In the infraclavicular region the ascent of the ribs during inspiration exhibits an undulatory movement which Hoover asserts is the resultant of two motions: the first brought about by the fact that the ribs increase in length from the first to the fifth, so that in their ascent a lower rib has a tendency to override an upper one; and the second produced by a rotation of the ribs in a sternovertebral axis, which gives rise to the so-called bucket-handle movement. In this case the maximum movement of the rib is in that portion farthest from its axis of rotation. On this latter point he apparently differs with Keith,[2] who points out that with the upper ribs there can be no rotation on the sternovertebral axis on account of the manner of articulation with the vertebræ. If we accept Keith's view, the second movement may be due to the fact that the transverse process on the vertebræ in which the convex ovoid process on the tubercle of the rib articulates, is tilted progressively backward as we descend the series, so that the angle at which the ribs are set in the spine progressively increases,

*From the Cardiorespiratory Laboratory, Medical Service, The Lakeside Hospital, Cleveland, Ohio.

making the axis on which the upper ribs rotate correspond to their necks. In either case the ascent of a lower rib differs from that of an upper one on account of the length of the rib and its axis of rotation on the vertebræ.

Hoover finds that in disease of the pleura, occlusion of the bronchus, inflammatory process of the lung, or displacement of the lung by an enlarged heart or pericardial sac, the extensibility of the lung is diminished before any increase in density is demonstrable, and that an impairment in the extensibility of the lung modifies the normal undulatory movement of the upper five ribs. For this reason a comparison of the undulatory movements in the two sides affords a very delicate method of determining the relative ventilation in the upper lobes. Any disparity in the undulatory movement on the two sides indicates diminished ventilation volume. The phenomenon is best observed from the right side of the patient. The examiner should use his left hand, placing the tip of the ring finger on the second rib at the midclavicular line, the tip of the third finger on the third rib midway between the midclavicular and the anterior axillary line, and the tip of the index finger on the fourth rib in the anterior axillary line. The patient is then instructed to make a moderately deep and rapid inspiration. The movement of each rib succeeds and exceeds that in the rib just above. A moderate impairment in the ventilation of the upper lobes causes the three ribs to move more in unison and the undulatory movement is diminished or lost, although the movement of the ribs may be considerable during inspiration. Hoover finds the test of very great practical use in the diagnosis of early pulmonary tuberculosis, since in this disease diminished extensibility of the lung can be demonstrated long before evidences of alteration in lung volume or density can be elicited.

Duchenne taught that the contraction of the diaphragm produces a separation of the costal margins, because in its descent it presses upon the abdominal viscera contained in its vault and rolls these forward, pushing out the lower ribs; and this teaching is current today. Hoover shows by experimental and clinical observations that this is not the most important or even a necessary factor in the production of the flaring of the costal margins seen in normal inspiration. He finds that the flaring of the costal borders is very prominent in paralysis of the diaphragm or in any condition in which the abdominal pressure is high and the arch of the diaphragm is correspondingly increased. On the other hand, paralysis of the intercostal muscles or any condition which depresses the arch of the diaphragm will result in the approximation of the costal borders to the median line and a decrease in the subcostal angle. The line of traction of the diaphragm is a straight one joining the central tendon with the edge of the ribs. When the diaphragm forms a well-defined arch, its traction is exerted at a disadvantage, and the external intercostals may have the mastery of the movements of the costal borders. In paralysis of the intercostal muscles, however, the diaphragm acts alone and the costal borders are drawn in. Likewise, when the arch of the diaphragm is depressed, the line of traction and the line of the muscular fibers of the diaphragm are more closely approximated. The diaphragm is then able to use its full force against the intercostal muscles, with the result that the costal border moves toward the median line. Depression of the diaphragm to such an extent that it actually

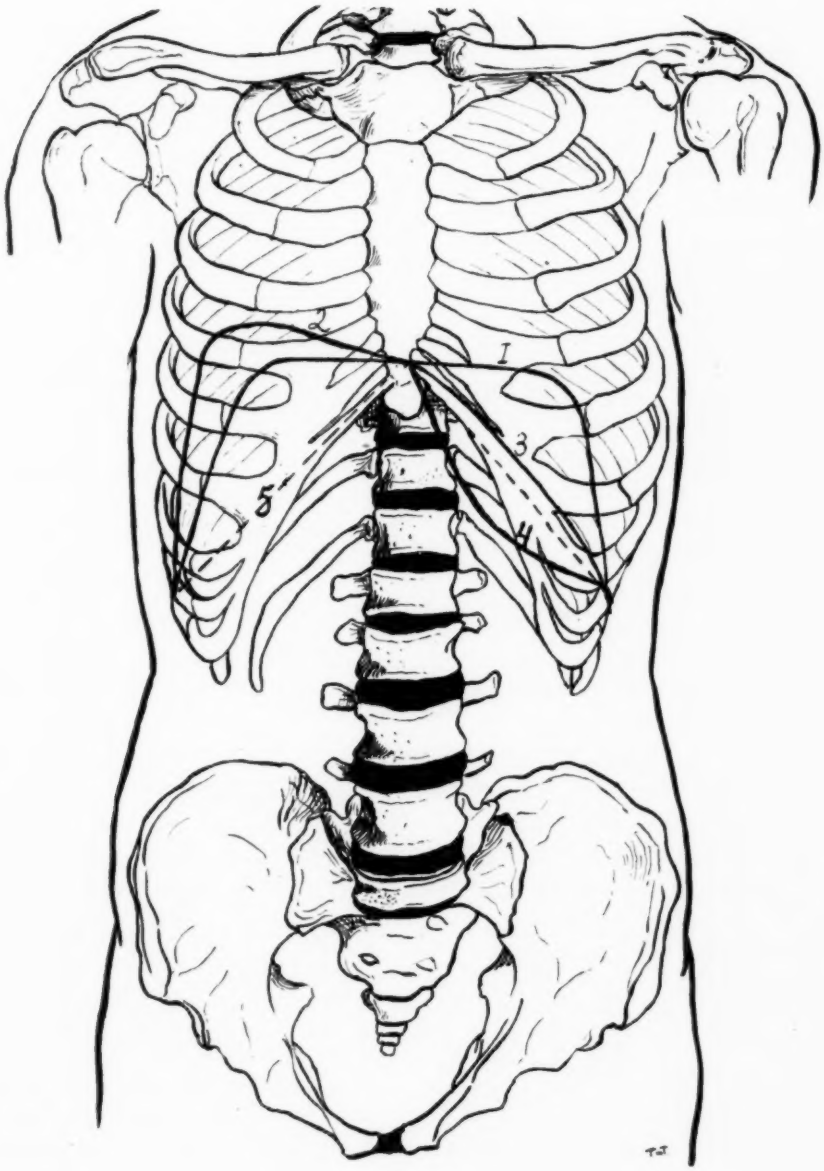

Fig. 1.

1. Normal position of diaphragm. Costal margins move out during inspiration.
2. High position of diaphragm. Normal outward movement of costal margins accentuated.
3. Low position of diaphragm. Costal margins move in during inspiration.
4. Very low position of diaphragm. Costal margins move out during inspiration.
5. Actual line of traction of diaphragm.

arched downwards should result in a flaring of the costal margins during inspiration, since the pull of its muscular fibers would no longer coincide with their line of traction as in the normal position. An example of such a condition was found in a patient with a massive empyema, in whom the movement of the costal margin was normal before the removal of any fluid. Partial removal of the pus resulted in the left costal margin being drawn towards the median line, and further removal brought about a return of the normal flaring. The experimental and clinical observations made by Hoover indicate that the contraction of the diaphragm approximates the costal margins, whereas contraction of the intercostal muscles produces lateral movements. When both act in unison, the resultant movement is dependent on which force is the stronger.

The movements of the margins can be determined by inspection or palpated by placing the thumbs on the costal borders and instructing the patient to take a somewhat deepened inspiration. Fig. 1 illustrates the movement of the costal margins when the diaphragm is low or high due to supra-or infraphrenic diseases, respectively.

The movements of the costal margins interpreted in this light at once serve to give much diagnostic information. Hoover calls special attention to the aid which their movements give in the diagnosis of pleurisy with effusion, empyema, and emphysema. Whenever the lowering of the sheet of the diaphragm is sufficient to give the diaphragm mastery over the intercostal muscles, the subcostal margin moves *in* rather than out during inspiration, and the angle made by them is decreased. Any disparity in the movements of the costal margin on the two sides likewise gives diagnostic data as to unilateral thoracic disease.

Anything which accentuates the arch of the diaphragm will cause the costal margins to flare more than normally. This fact serves to differentiate infra- and supraphrenic diseases such as hepatic enlargement, subphrenic abscess and empyema, pleurisy with effusion, or pericardial effusions. It likewise affords the only infraphrenic signs of diaphragmatic activation.

The curves of the different fibers of the diaphragm vary greatly; the arch is much less marked in that portion of the diaphragm which is attached to the costal margin near the median line than in that which joins the central tendon with the costal border in the axillary line. For this reason the anterior lateral part of the diaphragm requires less depression to give this part of the diaphragm a horizontal position than is required to accomplish the same for parts occupying a more lateral position. The heart overlies this part of the diaphragm, and most valuable and direct information can be obtained concerning the size of the pericardial sac and the various characteristics of the heart by careful study of the symmetry of movement of the two costal margins in their upper and median positions.

In any condition giving hypertrophy of the left ventricle—namely, chronic myocarditis, chronic valvular heart disease, or chronic nephritis—the costal margin on the *left* from the ensiform to the ninth rib is restricted in its outward movement during inspiration. In cases of acute dilatation of the right heart, auricle, or ventricle, the *right* costal margin from the ensiform to the ninth costal cartilage is restricted. In late valvular disease in which *both* sides are

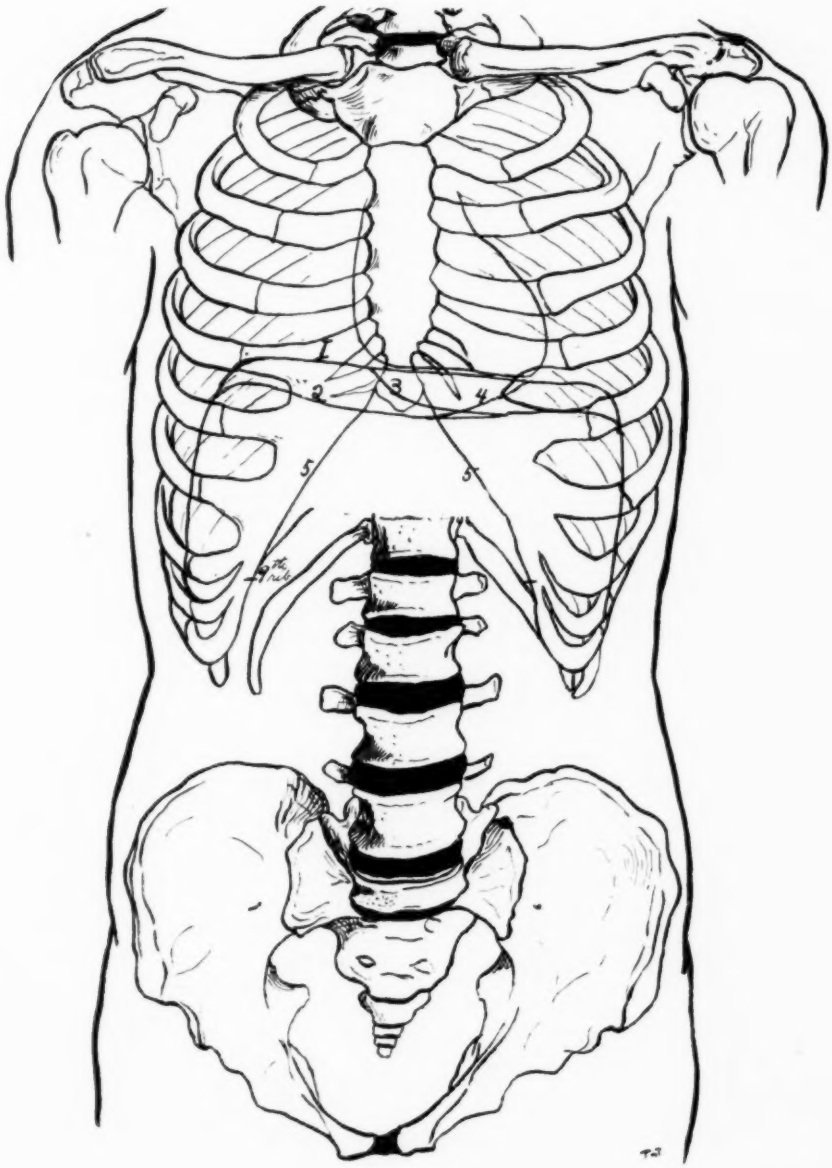

Fig. 2.

1. Normal position of diaphragm. Costal margins move out during inspiration.
2. Position of diaphragm in general cardiac enlargement. Costal margin from ensiform to ninth rib moves toward median line.
3. Position of diaphragm in left-sided cardiac enlargement. Left costal margin is fixed or moves in during inspiration.
4. Position of diaphragm in right-sided cardiac enlargement. Right costal margin is fixed or moves in during inspiration.
5. Costal margin.

involved, in general myocarditis, and when there is any increase in the size of the pericardial sac, there is a symmetrical restraint or even approximation of the costal margins during inspiration.

Restriction of the movements of the costal margin on one side must not be taken as absolute indication of cardiac enlargement, but must always be subjected to critical clinical observation. This was well illustrated by two cases which have come under my observation. Both were normal, healthy young men, and in good physical training. In both, the costal margin on the left from the ensiform to the ninth was restricted in its outward movement. Percussion gave the left border outside the nipple line in the sixth interspace. The upper border was found to be under the fourth rib. Auscultatory signs and blood pressure were normal. Fluoroscopic examination confirmed the percussion findings. The explanation of these phenomena lies in the fact that the hearts were occupying a lower and more transverse position than normal, and thus depressed the anterior leaf of the diaphragm. Fig. 2 shows position of the central leaf of the diaphragm in right and left and general cardiac enlargement.

BIBLIOGRAPHY

[1]Hoover: Arch. Int. Med., 1917, xx, 701.
[2]Keith: Further Advances in Physiology, Arnold, 1909, p. 182.

ERRATUM

In the article "A Clinical Method for Determining the Respiratory Exchange in Man," by R. G. Pearce, B.A., M.D., Cleveland, Ohio, in the April, 1918, issue of the JOURNAL, the following table (Table V) was omitted on page 431.

TABLE V

HEAT VALUE OF 1 LITER OF OXYGEN

Cals. for 1 Liter O_2 for Respiratory Quotients from .70 to .97.

0.70		0.75	4.708	0.80	4.770	0.85	4.831	0.90	4.892	0.95	4.954
0.71		0.76	4.720	0.81	4.782	0.86	4.843	0.91	4.904	0.96	4.966
0.72	4.673	0.77	4.733	0.82	4.794	0.87	4.855	0.92	4.917	0.97	4.979
0.73	4.683	0.78	4.746	0.83	4.807	0.88	4.868	0.93	4.930	0	
0.74	4.695	0.79	4.758	0.84	4.819	0.89	4.880	0.94	4.942		

The Journal of Laboratory and Clinical Medicine

VOL. III. MAY, 1918 No. 8

Editor-in-Chief: VICTOR C. VAUGHAN, M.D.
Ann Arbor, Mich.

ASSOCIATE EDITORS

DENNIS E. JACKSON, M.D. - - ST. LOUIS
HANS ZINSSER, M.D. - - - NEW YORK
PAUL G. WOOLLEY, M.D. - - CINCINNATI
FREDERICK P. GAY, M.D. - - BERKELEY, CAL.
J. J. R. MACLEOD, M.B. - - - CLEVELAND
ROY G. PEARCE, M.D. - - - CLEVELAND
ROGER S. MORRIS, M.D. - - - CINCINNATI
GERALD B. WEBB, M.D. - COLORADO SPRINGS
E. E. SOUTHARD, M.D. - - - - BOSTON

Contents of this Journal Copyright, 1918, by The C. V. Mosby Company—All Rights Reserved
Entered at the Post Office at St. Louis, Mo., as Second-Class Matter

EDITORIALS

Investigations on Shock at the Front

IF intensive concentration on one problem is the most certain way of insuring its solution, the pathogenesis of shock should not for much longer remain a mystery. A great many of the most eminent of American and British physiologists are at present engaged, some of them with experimental shock as observed in their home laboratories, and others with shock as observed at the front. As reports of their findings and experiences come to hand, it is important that they should be carefully studied by those of us who for one reason or another are not directly engaged in the actual observations. We must serve as an audience or tribunal, and thoughtfully study the evidence as it is presented, so that by friendly criticism and suggestion we may take a small though indirect share in the important work.

In a previous number of this JOURNAL, we reviewed a recently published series of papers by Y. Henderson and Haggard in which the claim is reasserted that shock depends upon a condition of so-called acapnia, and in that review it was pointed out that the evidence is insufficient and inapplicable to most forms of

shock. In the present article we would call attention to the much weightier contributions of Prof. W. B. Cannon and his collaborators working on actual cases of shock at the front, and recently reported in the *Journal of the American Medical Association*.[1-6] The investigations referred to are being conducted under the guidance of a special investigating subcommittee of the Medical Research Committee of Great Britain, the personnel of which includes such men as Bayliss, Starling, Sherrington, Cannon and Dale.

In one of these papers[5] Cannon sums up the evidence collected by himself and collaborators, and correlates it with that of previous workers, and it is to this excellent essay that we would particularly refer our readers. The first part concerns itself with a critical review of the existing hypotheses of shock. In criticising the acapnia theory Cannon gives what we believe to be an incorrect interpretation of Henderson's[7] views; to quote, "excessive breathing may greatly lower the CO_2 of the blood," so that "a protective compensation acidosis is developed to prevent the fatal apnea that might ensue from lack of stimulating H ions." The acidosis is assumed to exist because the CO_2 content of the plasma is reduced and since Cannon, like most others, has accepted Van Slyke's incomplete definition of acidosis as being a condition in which total plasma carbonates are reduced, it appears that he misinterprets Henderson's new hypothesis. This observer himself claims that during the time that the CO_2 is being "blown off" there is a relative excess of alkali in the blood and that this excess "passes out of the blood into the tissues." However this may be, Cannon clearly shows the acapnia hypothesis is quite untenable, and he points out that Porter's advocacy of the rebreathing of expired air by patients in shock, so that the CO_2 content of the blood may be raised, produces no more than temporary relief incident upon the stimulation of the respiratory and vasomotor centers. We shall consider Porter's observations more closely in a future article; suffice it here to state that Cannon does not support the treatment of shock by rebreathing expired air.

The possibility of suprarenal exhaustion is briefly dismissed as obviously untenable, and some space is then devoted to the erstwhile popular hypothesis of nerve cell exhaustion. Besides reviewing the already well-known evidence against this hypothesis, some further facts are cited, among them the observations by Cowell, one of Cannon's collaborators, indicating that the nervous system is functioning in normal fashion, since the intellect is clear, and the patient may retain his muscular vigor to such a degree that two men are necessary to hold him on the stretcher, although no pulse can be felt at the wrist. These facts indicate that there can be no exhaustion at least of the cells of the parts of the nervous system that are higher up than those controlling blood pressure, and if we take this along with the fact, very definitely established by Stewart, Pike and Guthrie some years ago, that the vasomotor center is the last to lose its function during acute cerebral anemia and the first to recover it when blood returns, we see how untenable is the exhaustion hypothesis. Whatever histologic changes may occur in the nerve cells in shock, it is pointed out, they are just as likely to be "the resultant of the low blood pressure as its cause." Persistent low blood pressure, accompanied by acidosis, which Cannon has found to exist, will no doubt ultimately, like hemorrhage itself, lead to secondary cell changes.

The possibility that the low blood pressure in shock is due to cardiac failure is readily dismissed, and attention is then directed to the only factor which remains to explain this low blood pressure; namely, deficiency in the amount of circulating blood. There are in general three prime factors responsible for the blood pressure: peripheral resistance, cardiac action, and amount of circulating fluid. If, as is the case in shock, the first two of these factors are practically unaffected, then we are driven by exclusion to conclude that it must be in the third, the loss of circulating fluid, that the abnormality exists. The conclusion of the earlier workers on the problem (Leonard Hill) must be reverted to, that in shock an animal bleeds into its own blood vessels, and the question that remains to be investigated is the exact location of the vessels. For obvious reasons we may at once dismiss the arteries from consideration. Concerning the veins, more especially those of the capacious splanchnic area, because a condition of experimental shock accompanied by great engorgement of the abdominal veins can be induced in animals by rough handling of the abdominal viscera, it has not uncommonly been assumed that a similar engorgement must also occur in surgical shock. Cannon, quoting interesting observations by Mann on the distribution of the blood after hemorrhage in normal and shocked animals, shows by computation that, if collection of the blood in the veins were really its cause, there would have to be in every case of shock a very evident engorgement of the abdominal veins, which however, as experience at the front testifies, is by no means the case. Neither can the extraabdominal veins serve as the trap for the lost blood, for, if this were so, then tight bandaging of the extremities and abdomen and placing the body in a slanting head-down position should relieve the condition, which however it does not do, although such treatment often gives temporary relief.

The conclusion is arrived at that *the blood stagnation must occur at a part of the vascular system that is beyond the sphere of vasomotor control; that is, in the capillary area.* This is confirmatory of the experimental findings of Mann, that the amount of blood that stagnates in the tissues of a shocked animal may be more than 50 per cent above that of a normal animal. By observations on man it is impossible to secure direct evidence of such capillary stagnation of blood; we must seek to prove it, therefore, by indirect means. But even this is most difficult to find, although the discovery made by Cannon and his coworkers that there is a marked increase in the concentration of the blood during its passage through the capillaries, indicates that something abnormal is occurring there. Briefly stated, it has been found that, whereas in normal persons the corpuscular count, the hemoglobin percentage, and the proportion of corpuscles to plasma (hematrocrite readings) are the same in blood taken from capillaries and veins, in shock the values are all greater in capillary blood. This discrepancy between capillary and venous blood is more marked in superficial (say, finger, etc.) than in deeper vascular areas. It indicates that the blood must become concentrated in the capillary areas.

These findings are of an opposite character to those following hemorrhage alone, in which both hemoglobin and corpuscles are reduced. In the cases of hemorrhagic shock observed at the front, the reduction of these values in the

capillary blood was distinctly less than in simple hemorrhage, being masked by the rise induced by the shock, although, as in the cases of uncomplicated shock, the venous blood was much more dilute than the capillary. An important point in the observations is that the venous blood was found to be of normal concentration; that is, the blood going to and coming from the capillary areas, is of the same measurable concentration—so that it must be while it is passing through the capillaries that the blood cells remain behind and accumulate so as to make the blood more concentrated. This does not of course prove that the blood has drained into the capillaries, causing a loss of circulating fluid and a holding back of blood from normal currency. It indicates rather that the holding back must be, not in the capillaries themselves, *but in the tissue cells or spaces.*

These observations are to be considered along with recent work by F. H. Scott,[8] which show that in laboratory animals there is a direct proportion between the arterial blood pressure and the hemoglobin content of the arterial blood, from which it is concluded that the increased pressure forces fluid out of the blood into the tissue spaces. Scott did not, however, compare the capillary and venous bloods in these particular experiments, because he found in other observations, as yet unreported in detail, that the percentage of hemoglobin is practically always identical in capillary and arterial (carotid artery) blood.

As an outcome of this work the question arises as to whether a maintained high arterial pressure in an animal not accustomed to it might cause such a drainage of fluid into the tissues that a concentration of corpuscles in the capillary blood, similar to that observed in shock, might occur, leading to a development of this condition after removal of the exciting cause of the high blood pressure. It is significant in this connection that many of the conditions which are followed by shock in experiments on animals cause a preliminary rise in arterial pressure (e.g., stimulation of afferent nerves.)

As to the causal relationship between low blood pressure and capillary stagnation of corpuscles, Cannon's articles are not quite clear. In one place he states that "besides a low blood pressure * * * there are other conditions that are favorable to capillary stagnation of the corpuscles," and in another, "that the capillary capacity is sufficient to contain the lost blood in shock, and that the chances of its doing so are greater the more concentrated the lost blood becomes." To the present writer it would appear that the fundamental cause for both the lowered pressure and the concentration of blood must be loss of fluid because of its leakage through the capillary walls into the tissues. This leakage is not great enough to produce edema or to make any measurable difference in the percentage of hemoglobin or of corpuscles in the arterial and venous blood of the part (which is not to be wondered at because of the large volume of moving blood at any given moment), but is yet quite sufficient to impede the free movement of blood in the capillaries because of increasing viscosity. Once such a hindering process is set agoing, it will become progressively more pronounced, for the increasing delay in movement will encourage ever larger amounts of fluid to exude; a so-called vicious circle will become established.

That the viscosity of the blood is greatly raised when the corpuscles in-

crease in number is a well-known fact, and, as Cannon points out, it is also raised by another of the conditions that are known to predispose to shock; namely, cooling of the surface of the body. Thus, the viscosity of the blood increases 3 per cent with a fall in 1° C. of temperature. Shock, as observed at the front, is much more liable to occur in men who have been exposed to extreme surface cooling, and the sweating and absence of shivering which accompanies it often lead to marked lowering of the mouth temperature, readings as low as 87° and 88° F. having been observed in shocked men. It has moreover been observed by one of Cannon's collaborators (Cowell) that as a wounded man becomes chilled his blood pressure falls, and as he is warmed it may rise again. The greater incidence of shock in cold, wet weather is also significant.

But there is yet another possible factor to be considered; namely, the acidosis which Cannon has shown to exist in shocked men. He found "in general that the lower the blood pressure, the lower the alkaline reserve," as measured by the Van Slyke method.[9] A similar reduction in alkaline reserve was also observed in cases of pure hemorrhage, but, so far as the results go, they tend to show that hemorrhage alone is not attended by as great a loss in the alkaline reserve as in shock (when the blood pressures are equally reduced.) The reduction in CO_2-combining power, except in very severe cases, was no greater than might be observed in a normal person after strenuous muscular exercise, but, whereas in the latter case the disturbance is temporary (oxidation quickly removing the organic acids responsible for it, and alkali reserves being called up from the tissues), in shock it steadily goes from bad to worse. Neither was it often observed to be so low as to cause evident stimulation of the respiratory center, although when the CO_2-combining power became very low (less than 30 per cent), the average respiratory rate was much greater.

As to the significance of the acidosis, Cannon, taking due cognizance of the work of L. J. Henderson and others, does not draw any hasty conclusions. He points out, for one thing, that the degree of lowering of the alkaline reserve of the circulating blood is probably not sufficient to cause a demonstrable increase in H-ion concentration (because of the buffer action of the blood). There can be little doubt that he is correct in this inference, and that the observations of Crile and others purporting to show an increase in C_H are untenable. But, although the blood itself may have no higher a C_H than normal, it may be quite otherwise in the tissue fluids, for it is here that the incompletely oxidized acids (lactic, etc.) will be produced. In the stagnant capillary blood, therefore, the C_H may be distinctly raised, and as the circulation through these vessels becomes more and more impeded, so will the acidosis of the stagnant blood increase. This local acidosis will be still further encouraged by the lowering of temperature, for it is known that the alkalinity of the body fluids decreases as the temperature falls.

Cannon sums up the possible effects which this local acidosis may bring about. The following are considered: (1) A dilatation of the smaller arterioles and capillaries. Although this local effect of acids on the smaller blood ves-

sels does occur, any increase in C_H of the blood as a whole would stimulate the vasoconstrictor center and so cause the richly innervated vessels, the arterioles, to become constricted, which they actually are in shock. (2) It will cause the heart to relax more in diastole and discharge less blood in systole. (3) It will increase the viscosity of the blood. Thus, in asphyxia the viscosity of the blood may be double that of normal richly oxygenated blood. This factor, as already explained, will operate to produce stagnation of corpuscles in the capillaries. (4) It will increase the size of the corpuscles (Hamburger), and so help to impede the capillary flow. Some evidence for this effect was secured by observing that "the capillary corpuscular volume was greater compared with the venous corpuscular volume than it should have been according to the rate of corpuscular and venous counts."

To sum up we may quote again: "It seems probable, therefore, that when acidosis is once established it would tend to continue the disturbances of the circulation which have been produced by other conditions."

Attention is directed to the importance of recognizing not only that many factors interact on one another in shock,—vicious circles, they are called,—but also that more careful distinction should be made among the several varieties often loosely classified under this general term. The term "exemia" is suggested as a suitable one to designate the shock due to a holding back of blood from normal currency. It seems to us, however, that, although there are signs that the obscurity surrounding this most mystifying of problems is being gradually dispelled by such researches as have been referred to in this article, we can not as yet see clearly enough the fundamental cause for the condition to justify the coining of a term which after all may refer to but one of its accompanying symptoms.

The researches emphasize the importance of keeping a threatened patient warm and taking means to prevent the development of acidosis. We shall, however, defer further reference to therapeutic measures to a future article.

BIBLIOGRAPHY

[1]Fraser, John, and Cowell, E. M., France: Clinical Study of Blood Pressure in Wound Conditions, Jour. Am. Med. Assn., 1918, lxx, 520.
[2]Cannon, W. B., Frazer, John, and Hooper, A. N., France: Some Alterations in Distribution and Character of Blood in Shock and Hemorrhage, ibid., p. 526.
[3]Cannon, W. B., France: Acidosis in Cases of Shock, Hemorrhage and Gas Infection, ibid., p. 531.
[4]Cowell, E. M., France: The Initiation of Wound Shock, ibid., p. 611.
[5]Cannon, W. B., France: A Consideration of the Nature of Wound Shock, ibid., p. 611.
[6]Cannon, W. B., Fraser, John, and Cowell, E. M., France: The Preventive Treatment of Wound Shock, ibid., p. 618.
[7]Henderson, Y., and Haggard, H. W.: Respiratory Regulation of the CO_2 Capacity of the Blood, Jour. Biol. Chem., 1918, xxxiii, 345.
[8]Scott, F. H.: Factors Influencing the Interchange of Fluid between Blood and Tissue Spaces. I. Blood Pressure. Am. Jour. Physiol., 1917, xliv, 298.
[9]Van Slyke, D. D.: Jour. Biol. Chem., 1917, xxx, 347.

—*J. J. R. M.*